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Vol. 32 July, 1955 No. 4

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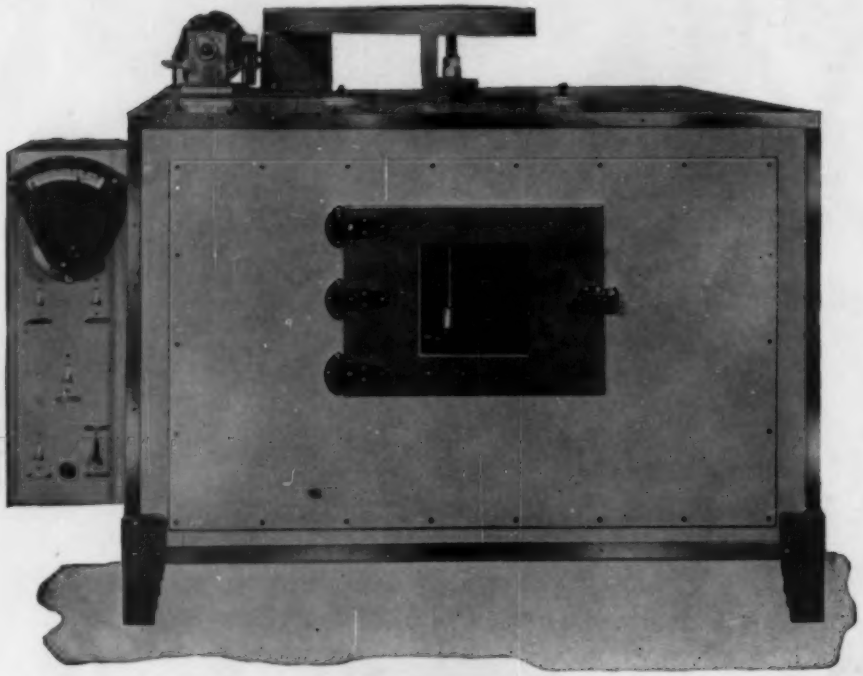

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The Vital Story of FARINA and PASTINA ENRICHMENT

by Science Writer

This article is one of a series devoted to the story of vitamin enriched or restored cereal foods; white bread and rolls, white flour, corn meal and grits, macaroni products, white rice, breakfast cereals and farina.

What is Farina? Sometimes called the "heart of the wheat," farina is wheat (other than durum varieties) which has been ground and bolted in granular form and from which virtually all of the bran coat and germ have been removed.

It is an excellent source of food energy, providing 370 calories per 100 grams. Its composition is largely carbohydrate (77.4 grams per 100 grams) with some protein value (10.9 grams per 100 grams) and low fat content.

This excellent, palatable food makes a fine dish for infants and children at any meal. For adults it is particularly good as a breakfast food when served with cooked or dried fruits. Being bland, it has a special place in the diets of the elderly and invalids of all ages.

Pastina Defined. Pastina is the product which is derived from durum wheat and with which egg yolks and sometimes other ingredients are processed. It, too, is an excellent food for babies and children and has a special place in adult diets, being used as a cereal or by being added to soups and broths.

Why Enrich Farina and Pastina? Because so much of the vitamin and mineral content of the wheat is contained in the germ and bran which must be removed to make farina and pastina, enrichment to restore important values is absolutely necessary.

New Enriched Farina Standards. The Food and Drug Administration of the U. S. Dept. of Health, Education and Welfare in June of 1955 changed the standards which all *enriched farina*, sold in interstate commerce, must meet. The new *maximum* and *minimum* levels (in milligrams per pound) are:

	Min.	Max.
Thiamine (vitamin B_1)	2.0	2.5
Riboflavin (vitamin B_2)	1.2	1.5
Niacin	16.0	20.0
Iron	13.0	*

*No maximum established

In addition to the above, the F. & D. A. allows the addition of other food elements at the manufacturer's option.

Among these are:

Vitamin D...250 U.S.P. units per lb.
Calcium ....500 milligrams per lb.

Enriched Pastina. Enrichment requirements for macaroni and noodle products, of which *pastina* is one, are as follows. All figures are in milligrams per pound and include allowances for losses which may occur in cooking. These are standards established for the consumer by the Food and Drug Administration.

	Min.	*Max.*
Thiamine (vitamin B_1)	4.0	5.0
Riboflavin (vitamin B_2)	1.7	2.2
Niacin	27.0	34.0
Iron	13.0	16.5

Again, the F. & D. A. allows manufacturers to add optional ingredients including vitamin D and calcium in the quantities noted below.

	Min.	*Max.*
Vitamin D	250	1000 (U.S.P. units per lb.)
Calcium	500	625 (mg. per lb.)

Enrichment's Simplicity. Enrichment is really a simple process. The enriching ingredients (vitamins and minerals) are added to the food during processing. The consumer then receives farina or pastina which equals or exceeds the values of the original wheat in vitally important vitamins and minerals.

Physicians, nutritionists, dietitians—and consumers—support enrichment enthusiastically. It is such an important factor in public health that not only farina and pastina and other macaroni products, but family white flour, white bread and rolls, corn meal and grits and white rice are enriched.

Duplicating Nature. The science of chemistry is so advanced these days that many of Nature's complex substances can be duplicated in the laboratory. This has happened with many vitamins. First, the chemical composition is learned. Second, the pure substance is isolated. Third, a "duplicate" is made by synthesis. And fourth, the laboratory techniques are extended to large scale operation. The manufactured duplicate is identical chemically and in biological activity with Nature's own product. A vitamin is still a vitamin regardless of its source. So efficient is large scale manufacturing that vitamins are sold at a lower cost than if they were extracted from natural sources.

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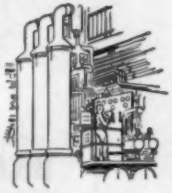

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CEREAL CHEMISTRY

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INFLUENCE OF TEMPERATURE ON STRUCTURAL RELAXATION IN BROMATED AND UNBROMATED DOUGHS MIXED IN NITROGEN[1]

C. J. DEMPSTER, I. HLYNKA, AND J. A. ANDERSON

ABSTRACT

The Brabender Extensograph has been used to study the behavior of bromated and unbromated doughs over the temperature range from 15° to 35°C. Unleavened doughs were mixed for three minutes in nitrogen and at constant absorption. Bromate concentration in the doughs was varied at each temperature from 0–120 p.p.m. at 15°C. to 0–40 p.p.m. at 35°C.

At a given temperature, structural relaxation in unbromated doughs tends to become more rapid with increasing reaction time. This effect is greater the higher the temperature. When bromate is present, structural relaxation at any one temperature becomes progressively slower with increasing reaction time. The change in dough properties increases with increasing bromate. These effects are also greater at higher temperatures.

The equation of an equilateral hyperbola referred to its asymptotes as axes has been used to characterize the relaxation curves. Changes observed in the hyperbolic relaxation parameters permit assessment of the rate of the bromate reaction for each bromate concentration at each temperature. The rate appears to be a linear function of concentration at each temperature.

Activation energies from about 11 to 19 kcal. per mole were calculated for structural relaxation in dough, the value increasing with the bromate concentration in the dough.

In this laboratory two separate studies of unleavened doughs involving rheological technics are in progress. On the one hand, a specially designed instrument, the relaxometer, is used to observe changes occurring in external stress required to maintain a constant deformation in a dough sample during the major portion of the relaxation process (2, 3). On the other hand, a conventional dough-testing instrument, the Brabender Extensograph, which records a load-extension curve at constant extension rate, is used to observe changes taking place in the properties of resting doughs after mixing or mechanical manipulation. Structural relaxation is the term employed to designate the latter type of change in dough properties.

[1] Manuscript received December 28, 1954. Presented at the Annual Meeting, Denver, Colorado, May 23-27, 1954. Paper No. 142 of the Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg, Manitoba, and No. 325 of the Associate Committee on Grain Research (Canada).

The structural relaxation technic has been used to obtain information about the reactions taking place in dough when chemical flour improvers are added (4, 5). A further study has now been made in which the relaxation behavior of bromated and unbromated doughs has been investigated at a series of different temperatures. In effect, the temperature study makes it possible to extend the time scale of observation: rapid changes are slowed down at lower temperatures and may thus be examined in greater detail, while in an analogous way slow changes are brought into the time scale of observation at higher temperatures.

An improved method of analysis of structural relaxation data has been developed since certain aspects of the exponential method of characterizing the relaxation curve made it desirable to seek another more adequate mathematical characterization. It now appears that the relaxation curve can be characterized more satisfactorily by a reciprocal or hyperbolic relation than by an exponential relation. This paper summarizes the results of the study of structural relaxation in dough at different temperatures and introduces the analysis of these structural relaxation data on the basis of a hyperbolic characterization.

Materials and Methods

The flour used in this study was an unbleached, improver-free, straight-grade sample commercially milled from a blend of Canadian hard red spring wheat. Thiamine had been added to the extent of 0.72 mg. per pound of flour. The protein content of the flour was 12.6%.

The technic used in studying the structural relaxation behavior of dough with the Brabender Extensograph has been described in detail previously (4, 5), and in terms of the present study may be outlined briefly as follows: unleavened flour doughs were prepared from 200-g. samples of flour at each of the temperatures 35°, 30°, 25°, 20°, and 15°C. The flour had been equilibrated in an atmosphere of nitrogen to reduce the oxygen content to a level which produced no effect on dough properties. The doughs were mixed for 3 minutes in an atmosphere of nitrogen in a specially designed G.R.L. mixer (6). The dough contained 1% of sodium chloride (flour basis). Potassium bromate in solution was added in varying amounts at the different temperatures as shown in Table I. A constant absorption of 62.5% was employed across the range of temperatures.

Flour doughs prepared at each of these temperatures were allowed reaction times varying from 0 to 4 hours. Doughs were then shaped on

TABLE I

RANGES OF TEMPERATURE AND BROMATE CONCENTRATION

Temperature	Bromate Concentration, p.p.m.						
	0	20	30	40	60	80	120
°C.							
15	x			x		x	x
20	x	x		x	x	x	
25	x	x		x	x	x	
30	x	x		x	x		
35	x	x	x	x			

the extensograph, clamped in dough holders, and allowed rest periods varying from 5 minutes to 180 minutes. After a desired rest period the dough was stretched by the extensograph and a load-extension curve (extensogram) was obtained. These extensograms were analyzed by reading the load supported by each dough at a constant sample deformation corresponding to a kymograph extension of 7 cm. A correction necessary to compensate for the downward displacement of the dough support was made as described in previous publications (4, 5). The structural relaxation curve is obtained by plotting the extensogram load, at constant sample deformation, against the rest period.

The sample deformation at which the extensograms of the present study were analyzed differs from that employed in our previous studies. Temperature has a marked effect on dough extensibility, the extensograms for the 35°C. doughs being the shortest. To permit analysis of extensograms for all temperatures, it was necessary to select a shorter sample deformation than had been used previously. Accordingly, a constant sample deformation corresponding to a kymograph extension of 7 cm. (corrected) was selected.

Preliminary exploratory experiments had shown that temperature had a marked effect on the changes in dough properties produced by the reaction of a given concentration of bromate. Consequently, it was not always possible to study the effect of a given concentration of bromate at all temperatures. For example, at the lowest temperature, 15°, a concentration of 20 p.p.m. bromate would produce only relatively slight effects even in a reaction time of 4 hours. Hence, at this temperature, in order to produce changes in dough properties of a considerable magnitude, relatively high concentrations of bromate were employed. On the other hand, at higher temperatures these higher concentrations of bromate would produce changes in dough properties of such magnitude as to be outside the range of measurement possible with the present setting of the extensograph. Therefore, at each tem-

perature, a range of bromate concentrations giving as wide a range of variation in dough properties as was conveniently measurable was employed.

Analysis of Structural Relaxation Data

A new method of characterizing the structural relaxation behavior of dough has been adopted, since it has now been found that structural relaxation curves may be closely approximated by a hyperbola. The equation of an equilateral hyperbola, referred to its asymptotes as axes, and having as asymptotes the lines

$$t = 0 \text{ and } L - L_A = 0 \text{ is } t(L - L_A) - C = 0. \quad (1)$$

A more useful linear form of this equation may be obtained by multiplying out and transposing terms, i.e.,

$$Lt = L_A t + C \quad (2)$$

where L = the load supported by a dough at a rest period, t;
L_A = the asymptotic load, i.e., a value of load which is approached at infinite rest period; and
C = the characteristic constant of the hyperbola, which will be called the "relaxation constant."

It is pointed out that the relaxation curve lies in the first quadrant. Hence the equation of the conjugate hyperbola in which the sign of C in equation (1) above is positive is of no interest. The reader should note that there is an inverse relation between the relaxation behavior and the characteristic relaxation constant; i.e., an increase in the relaxation constant occurs when relaxation becomes slower, and *vice versa*.

It can be seen that only two constants are needed to define the hyperbola and hence the relaxation curves: the relaxation constant C and the asymptotic load L_A. The method of calculating these constants is as follows. Equation (2) indicates that a plot of the product of load $\times$ rest period *vs.* rest period is linear, the intercept at the ordinate axis being equal to the relaxation constant C, and the slope being equal to the asymptotic load L_A.

A graphical example of the use of the hyperbolic relation in characterizing structural relaxation is given in Fig. 1. The left-hand portion of Fig. 1 shows a typical structural relaxation curve while the right-hand portion shows the product of load $\times$ rest period plotted against rest period. The straight line has been extrapolated to the ordinate axis to obtain the value of the relaxation constant C. The slope of the line gives the value of the asymptotic load, L_A.

The use of the hyperbolic relation to characterize structural relaxation in dough is preferred to the previously used exponential function,

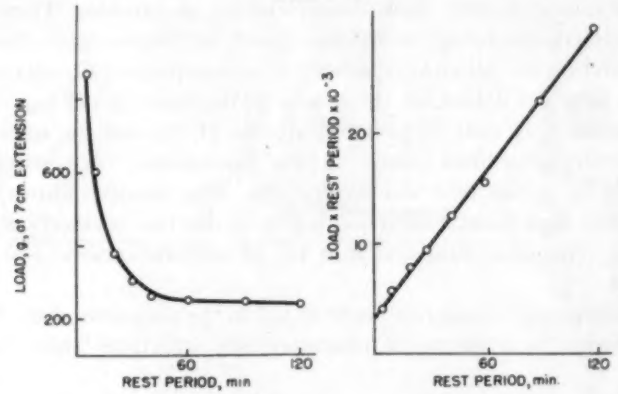

Fig. 1. Left, typical structural relaxation curve; right, linear transformation of the same curve.

for a number of reasons. It is not necessary, as was previously the case, to determine visually from the relaxation curve one of the characteristic constants and subsequently use it in the calculation of the other constant. On the basis of a hyperbolic characterization, both relaxation parameters are determined directly from the experimental data. Moreover, all of the experimental points on a given relaxation curve are included in these calculations. Accordingly, the rather large error introduced in the visual estimation of the steady state load does not present a problem in the hyperbolic characterization of structural relaxation.

In view of the above advantages, a large number of relaxation curves obtained in this and in previous studies were analyzed by both the exponential and hyperbolic methods of characterization. In some cases, the exponential transformation yielded good linear curves but in many cases the curves were bowed more or less markedly. Generally those curves showing marked deviation from linearity were bowed upwards, although a number of instances were found where the curves bowed downwards. On the other hand, it was found that the hyperbolic method of characterization yielded more exact linear transformations of structural relaxation data over a wide range of dough properties. Presentation of this mass of data does not seem warranted and will not be attempted.

However, a small statistically designed comparison was effected in an attempt to show that the hyperbolic characterization of structural relaxation provided a significantly better fit to experimental points than the exponential characterization. A number of the relaxation

curves obtained in this study were selected at random. These data were analyzed, assuming, on the one hand, an exponential characterization, and on the other a hyperbolic characterization. Correlation coefficients were calculated for these data in the form of the logarithmic transformation as used in previous studies (4, 5) and the hyperbolic transformation outlined above. These correlation coefficients were compared by means of z transformations. The results indicated that there was a significant difference between the two methods of characterizing relaxation data and that the hyperbolic method was to be preferred.

The foregoing considerations have led to the adoption of the hyperbolic relation as a means of characterizing structural relaxation in dough.

Results and Discussion

Structural relaxation curves were obtained at 35°, 30°, 25°, 20°, and 15°C. for doughs that were allowed reaction times from 0 to 4 hours and which contained varying amounts of bromate. Over 100 individual relaxation curves were obtained, but it is not necessary to present all these data. Sufficient primary data are presented to illustrate the major trends in the results, and in succeeding stages of the analysis of the results, data derived from almost all primary data are introduced. Accordingly, the final stages of the analysis show most of the derived analytical data for the various experimental conditions of this study.

Structural Relaxation in Unbromated Doughs at Different Temperatures. The upper portion of Fig. 2 shows relaxation curves for unbromated doughs obtained at 35°, 25°, and 15°C. Data for reaction times of 0, 2, and 4 hours only are shown; the data for reaction times of 1 and 3 hours, omitted to prevent crowding, fit in with the trends illustrated; data for a temperature of 30° were intermediate between those of 35° and 25°, while the data for 20° were intermediate between those of 25° and 15°.

The pattern for the structural relaxation behavior of unbromated doughs at 35° is similar to that reported in earlier studies at 30° (4). The load supported by the dough decreases quite rapidly at first with increasing rest period. With extended rest period up to 90 minutes, the load decays much more slowly. As the reaction time is increased, the relaxation curves fall progressively lower on the load axis. In addition, relaxation appears to be more rapid the higher the reaction time.

Changing the temperature modifies the structural relaxation be-

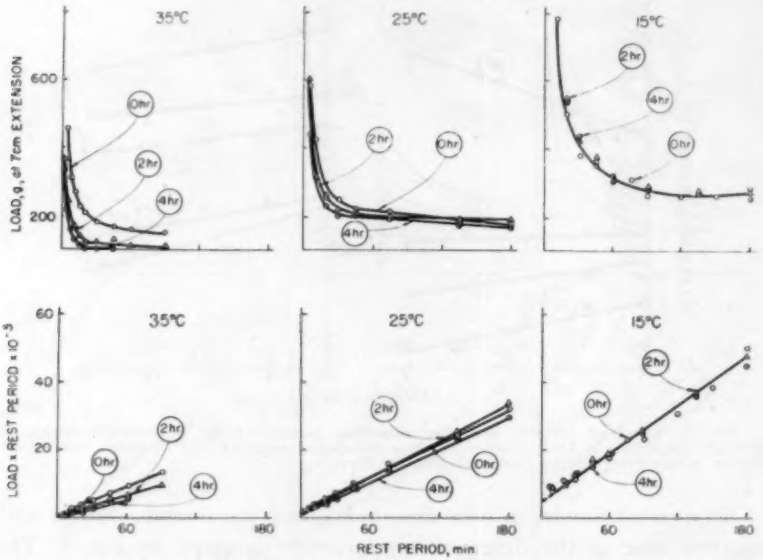

Fig. 2. Structural relaxation data for unbromated doughs: top, relaxation curves for temperatures 35°, 25°, and 15°C.; bottom, linear transformations of these data for calculating relaxation parameters.

havior of dough somewhat. At 25°, the 0 reaction time curve is located higher on the load axis than the corresponding curve at 35°. Moreover, relaxation appears to take place more slowly at 25° than at 35°. The extent of the downward displacement of the relaxation curves with increasing reaction time is less at 25° than at 35°.

At a temperature of 15°, the structural relaxation data are located higher on the load axis than at any of the other temperatures. The separation between the data for the various reaction times is not great and only one curve has been drawn. But it appears that the data for 0 reaction time are the lowest, while the data for the 2- and the 4-hour reaction times lie slightly higher on the load axis. Relaxation is slower at 15° than at any other temperature.

The experimental data are plotted as the product of load $\times$ rest period *vs.* rest period in order to calculate the hyperbolic relaxation constants. The lower portion of Fig. 2 shows these plots for the relaxation curves of this same figure. Regression lines were calculated for these data and are drawn in as solid lines in the region of experimental rest periods and as dashed lines where the curve is extrapolated to intercept the ordinate axis. This intercept gives the value of the relaxation constant C while the slope of the line gives the value of the asymptotic load L_A.

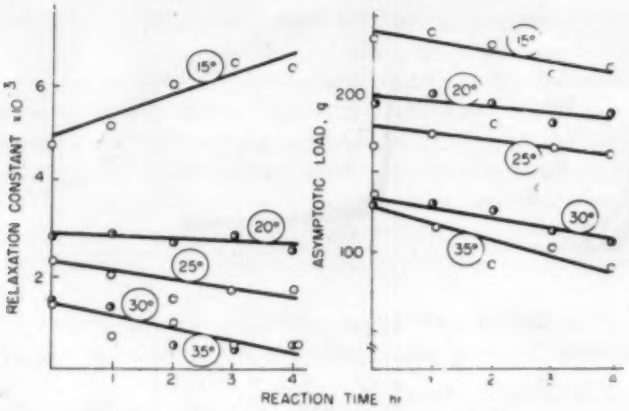

Fig. 3. Effect of reaction time on relaxation parameters for unbromated doughs at different temperatures: left, relation between relaxation constant and reaction time; right, relation between asymptotic load and reaction time.

An over-all picture of how these relaxation parameters change with reaction time at the different temperatures is given in Fig. 3. The relaxation constant, plotted on the left in Fig. 3, changes more or less linearly with reaction time at all temperatures. At the higher temperatures, the relaxation constant decreases with increasing reaction time; i.e., dough relaxes more rapidly. As the temperature is lowered the rate of change of the relaxation constant gradually decreases until finally at 15° there is an increase in this parameter.

The asymptotic load, plotted on the right in Fig. 3, decreases with reaction time at all temperatures. The rate of change does not seem to vary in a regular manner with a change in temperature.

The curves of Fig. 3 show that there is, in general, a progressive increase in the relative value of the relaxation constants as the temperature is lowered, i.e., structural relaxation in dough takes place more slowly the lower the temperature. Similarly there is a progressive increase in the relative value of the asymptotic load as the temperature is lowered. These changes are due in part to the fact that a constant absorption was employed in this study so that dough consistency varied with temperature.

Structural Relaxation in Bromated Doughs at Different Temperatures. The changes in the structural relaxation behavior of bromated doughs as temperature is varied are illustrated by presenting data for a bromate level of 40 p.p.m. The upper portion of Fig. 4 shows relaxation curves for 40 p.p.m. bromated doughs at different reaction times at temperatures of 35°, 25°, and 15°C.

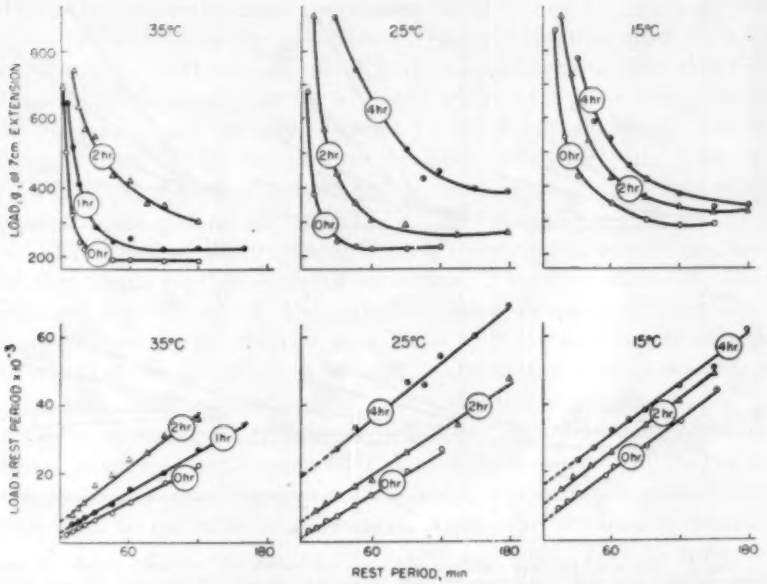

Fig. 4. Structural relaxation data for doughs containing 40 p.p.m. potassium bromate: top, relaxation curves for temperatures 35°, 25°, and 15°C.; bottom, linear transformations of these data for calculating relaxation parameters.

At 35°, relaxation curves for 0-, 1-, and 2-hour reaction times are presented. With increasing reaction time, relaxation becomes slower and in addition the curve is located progressively higher on the load axis. This behavior is similar to that reported previously for a single bromate level in dough at 30° (4). As the temperature is decreased to 25° and then to 15°, the extent of the change in dough properties produced by the reaction of bromate becomes less. An effect similar to that observed for unbromated doughs is the progressive rise in the 0 reaction time relaxation curve as the temperature is lowered.

The relaxation curves for bromated doughs were analyzed in the same manner as outlined above. The lower section of Fig. 4 shows the plots of the product of load $\times$ rest period *vs.* rest period for the relaxation data for doughs containing 40 p.p.m. potassium bromate. The calculated regression lines for these data are shown. The changes in the relaxation constant C and the asymptotic load L_A are more apparent than was the case for the unbromated doughs. At a given temperature, both the relaxation constant and the asymptotic load increase with reaction time. The higher the temperature, the greater the extent of such changes.

To investigate how dough properties change with time for the

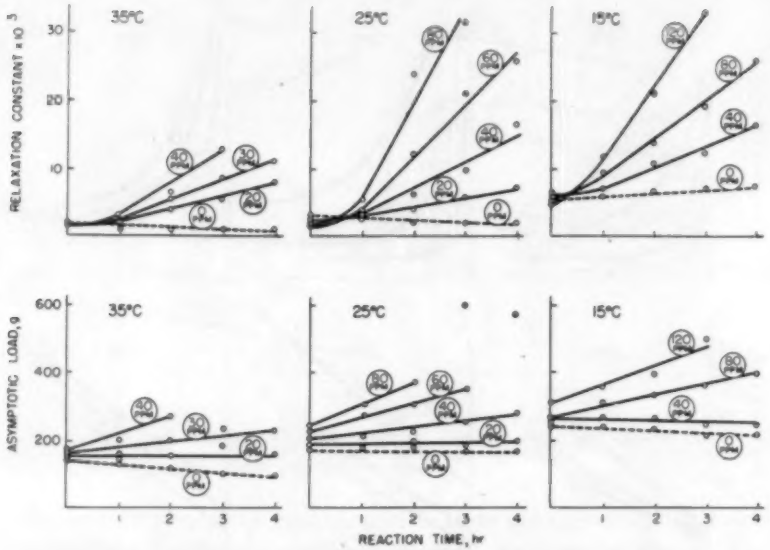

Fig. 5. Effect of reaction time on relaxation parameters for bromated doughs at temperatures 35°, 25°, and 15°C.: top, relation between relaxation constant and reaction time; bottom, relation between asymptotic load and reaction time.

different bromate levels at each temperature, the relaxation parameters are plotted against reaction time, as shown in Fig. 5. The upper portion of this figure shows the plot of relaxation constant *vs.* reaction time for each bromate level at temperatures of 35°, 25°, and 15°C., while the lower portion of the figure shows the plot of the asymptotic load *vs.* reaction time. To make the picture complete, the data for unbromated doughs are also included and are represented by dashed lines.

Appreciable changes take place in the relaxation parameters with reaction time, depending upon the bromate concentration and temperature. For a given bromate concentration at any temperature, the relaxation constant increases with time. Beyond a reaction time of about 1 hour, the relaxation constant appears to increase linearly. Increasing the bromate concentration at any one temperature increases the rate of change of the relaxation constant.

A somewhat similar picture is shown by the plot of the asymptotic load against reaction time. In most cases the asymptotic load increases linearly with reaction time. For a given temperature, the higher the bromate concentration the more rapid is this change.

It is noted that in the asymptotic load-reaction time plots there were four cases where a marked deviation from linearity was observed.

This occurred for bromate concentrations of 60 and 80 p.p.m. at both 25° and 20°C. Only the point for the longest reaction time for each concentration and temperature seems to contribute to this deviation from linearity. This is shown in Fig. 5 where at 25° the 3-hour point for 80 p.p.m. and the 4-hour point for 60 p.p.m. do not lie on the straight lines. It may be that this deviation is a real result, but until it is confirmed these points which do not fit in with the apparently general trend will be neglected.

The curves of the foregoing figures show that although the basis of the method of analysis of structural relaxation data has been changed, the information about the changes in dough properties which can be derived from the raw data fit in with the patterns established in our previous publications (4, 5). The reaction of a given amount of potassium bromate produces an essentially linear increase in the relaxation constant C. This corresponds to the previously reported linear change in the exponential relaxation rate constant except for the sign which is a consequence only of the type of mathematical function used to characterize relaxation. Again, the reaction of bromate produces a linear change in the asymptotic load. This in turn corresponds to the previously reported linear change in the steady state load. It should be added that the calculated asymptote has never been found to exceed the steady state load read from the relaxation curve.

Regardless of the method of characterizing the structural relaxation curve, the mathematical relaxation parameters summarize separately and in single figures the behavior of the dough at a given reaction time. Chemical reaction in the dough produces changes in the physical properties which are mirrored in the characteristic constants.

The rate of change of the relaxation constant C is a measure of the rate of change of dough properties and accordingly is, in effect, a measure of the rate of reaction of potassium bromate in the dough. Thus the curves of Fig. 5 where the relaxation constant C is plotted against reaction time show the effect of concentration on the reaction of bromate at the different temperatures. The curves are linear, at least beyond a reaction time of 1 hour, and the rate of the bromate reaction, i.e., slope of the curve, is readily calculated for these conditions.

In like manner, the rate of change of the asymptotic load with reaction time provides a measure of the rate of the bromate reaction. The curves of the lower portion of Fig. 5 provide an independent estimate of the effect of concentration on the reaction of bromate at the different temperatures. Data for the rates of reaction of potassium bromate in dough at the various temperatures and concentrations calculated by both of the above methods are recorded in Table II.

TABLE II

RATES OF BROMATE REACTION AT VARIOUS BROMATE CONCENTRATIONS AND TEMPERATURES

Bromate Concentration	Rate of Bromate Reaction				
	Calculated as Slope of C vs. Reaction Time Plot				
	35°C.	30°C.	25°C.	20°C.	15°C.
p.p.m.					
0	−0.18	−0.30	−0.20	−0.08	0.45
20	2.02	1.96	1.52	1.02	...
40	4.95	5.78	5.26	3.64	3.02
60	...	9.40	7.44	7.56	...
80	...	...	13.1	9.02	5.40
	Calculated as Slope of L_A vs. Reaction Time Plot				
0	−13.5	− 7.0	− 2.4	− 4.2	− 7.0
20	4.4	1.8	− 2.2	− 2.4	...
40	50.5	29.6	18.9	1.9	− 2.4
60	...	83.7	41.3	24.6	...
80	...	...	65.0	59.6	32.0

There are at least two major effects involved in the changes taking place in dough properties as a consequence of varying temperature and bromate concentration: the effect of temperature on the reaction of bromate, and the effect of temperature on dough consistency. Unfortunately, it does not seem possible with the data of this study to separate these two effects analytically. This imposes limitations on the rate data of Fig. 5. Nevertheless, for a given temperature, the slope of the curves of Fig. 5 provides a measure of the relative rate of the reaction of bromate. For each temperature, the reaction rate calculated from the relaxation constant data increased essentially linearly with increasing bromate concentration. This is in agreement with a previous observation at 30° (4). On the other hand, for data derived from the asymptotic loads, the reaction rate at any one temperature did not seem to be a linear function of concentration. Rather, the rate appears to increase somewhat more rapidly with concentration.

Activation Energy for Structural Relaxation. An attempt was made to evaluate the activation energy for structural relaxation employing a method widely used in stress-relaxation of polymers (1, 2). The relaxation curves for all doughs allowed 0 reaction time were translated along the y- or load-axis so that the line $y = 0$ became the asymptote; i.e., for each curve the particular value of L_A was subtracted from each experimental load value. When the corrected loads supported by doughs containing a given amount of bromate were plotted against the logarithm of rest period, it was found that temperature did not

appreciably alter the shape of the curves. For a given bromate level, the curves at all other temperatures can be superposed on the 25° curve by multiplying the rest periods by an appropriate factor. Apparent activation energies for structural relaxation can be calculated from the magnitudes of these lateral shift factors. Table III lists the

TABLE III

LATERAL SHIFT FACTORS AND ACTIVATION ENERGIES FOR STRUCTURAL RELAXATION IN UNLEAVENED DOUGH

Bromate Concentration	Lateral Shift Factor					Activation Energy
	Temperature, °C.					
	15	20	25	30	35	
p.p.m.						*kcal/mole*
0	0.45	0.70	1	1.5	1.8	11.9
20	...	.75	1	1.6	1.6	10.5
40	.35	.73	1	1.5	1.3	11.9
60	...	.48	1	1.2	...	15.5
80	0.33	0.65	1	...	...	19.1

amount of lateral shift for the different temperatures at each bromate concentration. From plots of the logarithm of this lateral shift against the reciprocal of absolute temperature, values for the activation energy for structural relaxation at each bromate level were calculated. There was some scatter of points in the individual Arrhenius plots from which these values were calculated, but there was no pronounced suggestion of a deviation from linearity. The calculated values for the activation energy of structural relaxation show a tendency to increase with increasing bromate concentration, the values ranging from about 11 to 19 kcal/mole. It is pointed out that the magnitude of these values is of the same order as values for the activation energy of stress relaxation in dough as reported by Cunningham and Hlynka (2).

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INVESTIGATION OF PROTEOLYTIC ENZYMES BY A GELATIN VISCOSITY TECHNIC[1]

R. B. KOCH AND C. G. FERRARI[2]

ABSTRACT

A gelatin viscosity method, originally developed by Northrop to measure pepsin activity, has been applied with appropriate modifications to a study of proteolytic enzymes of interest in baking. The observed effects of fungal proteinases on bread doughs correlate better with pH optima when measured by the above method than by the Ayre-Anderson hemoglobin technic. The simplicity and accuracy of the gelatin viscosity method recommend it as a research and a control method.

When applied to proteinases derived from *Aspergillus oryzae* and a commercially available fungal product (Rhozyme P-11), the pH optima obtained by the present method were quite broad, with a median about pH 6.9. However, the optimum of Rhozyme P-11 given by the formol titration procedure with a hemoglobin substrate was sharp at pH 4.1. Another commercially available fungal proteinase preparation (Lipase B) had a different type of pH activity curve than the above, in that it had a sharp optimum at pH 4.1 followed by a plateau of fairly constant but much lower activity between pH 6.2 and 8.1. These latter results suggest the presence of two different proteolytic enzymes.

Three plant proteinases had pH optima between 4.1 and 4.7, while an animal proteinase had a broad optimum in the slightly alkaline pH range.

The gelatin viscosity procedure showed very little if any effect of salt on proteinase activity. The observed effect of salt in bread doughs appears likely to be due to its action on the availability of the substrate to enzyme attack rather than to an inhibition of proteinases themselves.

As the use of proteinase products by bakers for decreasing mixing time and improving dough and bread properties has become so widespread, it was deemed desirable to have a simple, rapid, and accurate method for measuring their relative activity by a procedure that can be correlated with baking results. Examination of a number of existing methods showed that most were either too time-consuming or inaccurate, or did not correlate with observed effects. Among the methods considered, the gelatin-viscosity method of Northrop and Hussey (19)

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seemed to offer the greatest possibilities for developing a convenient research and control method. As suggested by Hildebrand (5) and Johnson and Miller (8), the physico-chemical rather than chemical methods may give better correlations with the dough modification caused by proteolytic enzymes.

Procedures for measuring proteolytic activity in different products have been reviewed by Hildebrand (5, 6) and more recently by Bowlby *et al.* (2). Hildebrand (5), in his original comparison of methods, concluded that the gelation-rate method of Landis and Frey (11) and the viscometric method of Koch *et al.* (10), which has since been modified (9), gave similar results, while the formol titration method gave substantially different results. A suggested possible explanation of the difference between the methods was that the physico-chemical methods measured proteinase activity whereas the formol titration method measured chiefly the action of dipeptidase and/or polypeptidase.

In later work, Hildebrand (6) found that the gelation-rate method and the Ayre-Anderson (1) hemoglobin method, which has been modified by Miller (17), gave essentially the same results in investigations of the proteinase activity of malted wheat flour. Miller (17) made a critical study of the Ayre-Anderson method and introduced several modifications. Bowlby *et al.* (2), in their comparison of proteolytic methods for measuring proteinase activity of products from various sources, concluded that the modified Ayre-Anderson method, the bromsulphalein method, and a formol titration method, all using hemoglobin as a substrate, gave results essentially similar to those obtained with the farinograph method of Johnson and Miller (7, 8).

In their comparison of methods, Bowlby *et al.* apparently did not investigate the gelatin-viscosity method developed by Northrop and Hussey (19, 20) for measuring pepsin activity. Northrop (19) pointed out that this method has the advantage of rapidity and accuracy and that it is a most convenient and reproducible procedure. Northrop also points out that there is some question as to the physical significance of the method and especially its relation to chemical changes when pepsin or trypsin was used as the enzyme source.

Nevertheless, Laufer (12) recommended the gelatin viscosity method for the measurement of proteinase activity of barley malts. Laufer (13) and Koch *et al.* (9) published modifications of the Northrop gelatin viscosity method for the determination of proteolytic activity of malt. Reid (22) used a viscometric method for the investigation of fungal pectinase and found that the method could be used for the assay of fungal proteolytic activity by substituting gelatin as a substrate. More recently Dworschack *et al.* (4) and Matsushima (14, 15) used the ability

of mold proteinase to hydrolyze and liquefy gelatin as a means of studying the production of proteinase enzymes by molds, particularly *Aspergillus flavus-oryzae*. Matsushima (15, 16) found that the gelatin-liquefying ability of molds was the most convenient method for selecting useful strains for manufacturing soy sauce. Dworschack *et al.* (4) found that the proteinase from *Aspergillus flavus-oryzae* hydrolyzed gelatin most rapidly at pH 7.5, much less at pH 5.0, and not at all at pH 2.5. Pechmann (21) in his studies on substituted casein as a substrate found that, unlike yeast proteinase and papain, the Aspergillus proteinases were not sensitive to oxidation or to heavy metals and possessed a broader pH activity range. Simonart and Chow (23), in their studies on the chromatographic separation of amylase and proteinase from *Aspergillus tamarii*, used ammonium sulfate buffered at pH 6.5 as the irrigating solvent.

The results cited are quite different from those found by Johnson and Miller (7, 8), using the farinograph technic and the Ayre-Anderson hemoglobin method (17). They reported that two commercially available fungal products (Rhozyme P-11 and Rhozyme S) showed sharp pH optima at 4.12 which decreased rapidly on either side of the optimum point. Miller and Johnson (18) also concluded from their studies of amylase and proteinase in baking that sodium chloride acts as a proteolytic enzyme inhibitor. However, Matsushima (14) used 1% sodium chloride solution to extract the proteolytic enzymes from mold cultures in order to carry out his studies on the gelatin-liquefying ability of mold proteinase. The farinograph method of Miller and Johnson (8, 18), while giving good agreement with the hemoglobin method, showed that the fungal proteolytic enzymes had little activity at pH 5.2. These authors (8) state that pH 5.2 could have been selected, but the sensitivity of the farinograph method at this pH was too low to be practical. Results with the farinograph and hemoglobin methods are contrary to baking experience where minute quantities of concentrated fungal enzyme preparations of the order of 0.006% (flour basis) added to sponge doughs are sufficient to ruin a dough for breadmaking purposes. Since the pH of a sponge dough changes from approximately 5.5 to 4.8 throughout the fermentation period, one would expect from the results of the farinograph and hemoglobin methods that there would be very little proteinase activity until possibly the latter part of the sponge fermentation period.

Consequently, it was decided to investigate gelatin as a substrate for proteolytic enzymes, not only because of the simplicity and accuracy of the viscometric technics, but also because it might show a better correlation between the pH activity curves and the results ob-

tained in commercial baking practice. However, the hemoglobin method was also used to check the activity of a fungal preparation and compare it with results reported in the literature.

Materials and Methods

Proteolytic preparations examined included papain, malted barley, a crude sample of lipase,[3] soybean flour extracted with hexane, and six samples of fungal enzyme concentrates: Rhozyme P-11, Rhozyme S, Lipase B, Rhozyme A-4,[4] Fungal Enzyme A, and Fungal Enzyme B.[5] Four of the fungal concentrates, Rhozyme S, Rhozyme A-4, and Fungal Enzymes A and B, are prepared from *Aspergillus oryzae* and are permitted ingredients in breadmaking by the "Definitions and Standards of Identity" for bread.

The formol titration procedure described by Bowlby *et al.* (2) was followed to check the fungal proteinase activity on a hemoglobin[6] substrate. Twenty-five ml. of a 5% hemoglobin solution were mixed with 2 ml. of enzyme solution and 25 ml. of 0.1 *M* sodium acetate buffer at pH 4.7 (all solutions attempered to 40°C.). The mixture was held in a constant-temperature water bath at 40°±0.01°C. for the desired time, then the reaction stopped by the addition of 5 ml. of 60% trichloroacetic acid solution. The resulting precipitate was removed by filtration through Whatman No. 4 filter paper. A 20-ml. aliquot of filtrate was mixed with 50 ml. of distilled water and the pH of the mixture adjusted to 7.0 with standard sodium hydroxide using a line-operated Bechman pH meter. After the mixture was brought to pH 7.0, 15 ml. of neutralized 37% (approximately) formaldehyde solution were added and the mixture was again brought to pH 7.0 with 0.05 *N* NaOH. Reagent blanks were run at each pH level by substituting 2 ml. of distilled water for the 2 ml. of enzyme solution.

The gelatin viscosity method of Northrop (19) was essentially adopted for studying the effect of proteinase activity on a gelatin substrate. Ostwald viscosimeters were selected which had outflow times of between 38 and 50 seconds for that portion of a 5-ml. aliquot of distilled water held in the upper bulb of the pipette. The substrates for enzyme action were buffered (0.185 *M*) 5% gelatin solutions. The buffer solutions of Clark and Lubs (3) and McIlvaine (3) as well as sodium acetate-acetic acid solutions were used for keeping the pH of the reaction mixture at a constant level.

The reaction mixture was prepared by combining 1 ml. of enzyme

[3] Pancreatic lipase from General Biochemicals, Inc.
[4] Rhozyme P-11, Rhozyme S, Rhozyme A-4, and Lipase B from Rohm & Haas Company.
[5] Fungal Enzymes A and B from Wallerstein Company, Inc.
[6] Difco-Bacto hemoglobin.

solution (or 1 ml. of distilled water for the blank) and 10 ml. of 5% gelatin solution in a 50-ml. Erlenmeyer flask in a constant temperature bath, all solutions being previously attempered to 40°C. A 5-ml. aliquot of the reaction mixture was then pipetted into an Ostwald viscosimeter which was hooked on the side of a glass jar constant-temperature bath set at 40°±0.01°C. The change in relative viscosity of the reaction mixture was followed by periodically measuring with a stopwatch the outflow time of the solution from the upper bulb of the Ostwald viscosimeter.

$$\text{Relative viscosity} = \eta_r = \frac{\text{outflow time gelatin solution (seconds)}}{\text{outflow time distilled water (seconds)}}$$

Zero time for the reaction was taken at the instant the gelatin was added to the enzyme solution. The reaction time listed in the tables is the time at which the outflow began. A more accurate reaction time can be obtained by adding one-half of the outflow time to the stated reaction time. A measure of proteinase activity was taken as the difference in relative viscosity between the blank gelatin solution and the gelatin solutions containing the added enzyme after a selected time interval. In the enzyme solutions containing a high amount of solids (as in soybean extracts), the initial viscosity had to be estimated because blank viscosities were too low and heating caused precipitation of proteins in the extract.

In order to compare the relative strength of different proteinase preparations, it is merely necessary to find the concentration of each preparation that will produce a given decrease in relative viscosity at a constant pH and temperature in a specified time interval.

Data were obtained for various reaction times from 5 to 150 minutes. In comparing the enzyme activity of various proteinase preparations, a reaction time of 90 minutes was selected because it afforded greater accuracy than shorter intervals, and was not unreasonably long.

Results and Discussion

Effect of pH on the Digestion of Hemoglobin by Fungal Proteolytic Enzymes. With the use of hemoglobin as a substrate and the formol titration procedure for determining nonprecipitable nitrogen, results quite similar to those reported by Johnson and Miller (8) and Miller (17) were obtained. The effect of pH on the activity of the proteolytic enzymes of Rhozyme P-11 is shown in Fig. 1. The optimum occurred at pH 4.1 and was quite sharp.

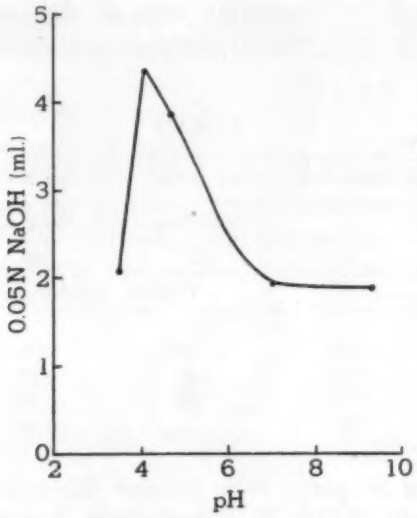

Fig. 1. Effect of pH on the proteinase activity of Rhozyme P-11 with a hemoglobin substrate. (Reaction time 2 hours, at 40°C. Enzyme solution 10 mg. per ml.)

With increasing pH the activity fell off rapidly up to pH 7.0 and then showed only a slight decrease from this point to pH 9.3. It is over the latter range that the proteinase activity shows a broad optimum when gelatin is used as the substrate, as will be shown later. The blank determination for the hemoglobin method was much higher at pH 7.0 than at any other pH measured; the value was 1.15 ml. of 0.05 *N* sodium hydroxide for a 15-minute incubation period at 40°C. At all other pH values investigated, the blank averaged about 0.35 ml. of 0.05 *N* sodium hydroxide including the experiment run at pH 9.3. The reason for the high blank at pH 7.0 was not investigated.

Effect of Temperature on Gelatin Viscosity and Rate of Enzyme Action. As pointed out by Northrop (19) the viscosity of gelatin solutions increases with time at 30°C. This effect was also noted in the present experiments and consequently relative viscosity values for enzyme activity at 30°C. were considered unreliable. There was a large difference in blank viscosities between 35° and 40°C. At 40°, 45°, and 50°C. blank viscosity readings became progressively lower, but the difference was not nearly so large as it was between 35° and 40°C. A slow decrease in blank viscosity with time at all temperatures was also noted. This decrease was taken into account in calculating the relative viscosity of the enzyme gelatin mixture. The results of blank determinations of relative viscosity at various temperatures are

given in Table I. In consecutive runs on the same blank, it was found that the relative viscosity calculations agreed within ± 0.02 η_r unit.

TABLE I

EFFECT OF TEMPERATURE AND TIME ON RELATIVE VISCOSITY OF BLANK GELATIN SOLUTIONS (4.55%) AT pH 4.7

Temperature	Reaction Time (Minutes)			
	5	30	60	90
	Relative Viscosity			
°C.				
35	7.15	7.06	6.98	6.90
40	5.33	5.30	5.28	5.27
45	5.13	5.10	5.09	5.08
50	4.82	4.75	4.74	4.70

The effect of temperature on enzyme (Rhozyme A-4) activity is shown graphically in Fig. 2. These results helped in choosing a temperature for following proteolytic activity on gelatin.

A temperature of 40°C. was considered more satisfactory than 45° or 50°C. as there was less enzyme inactivation at the lower temperature. A possible explanation for the change in slope of the curves between 35° and 40°C. from the 60-minute to the 90-minute interval is

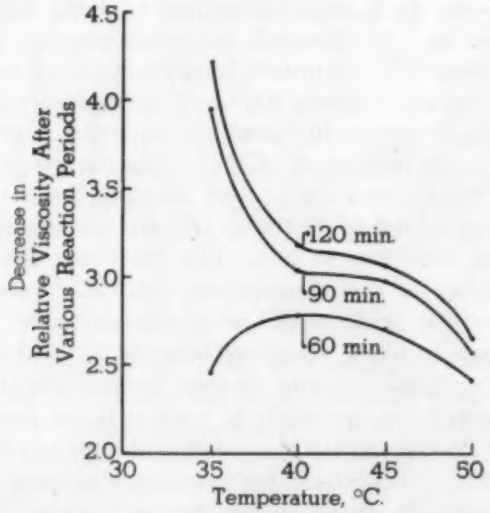

Fig. 2. Effect of temperature on the proteinase activity of Rhozyme A-4 on gelatin. Reaction mixture: 4.55% gelatin, 1 mg. Rhozyme A-4 per 11 ml.; pH 4.6.

that temperature would not have as much effect on the viscosity of the enzymatically split products of gelatin as it does on the unsplit gelatin molecules. Consequently, a high initial viscosity at 35°C. would falsely contribute to the apparent proteinase activity.

Effect of Buffer on Proteinase Activity.

A. Buffer concentration. The results of this investigation, shown in Table II, indicated that between 0.05 and 0.2 *M* the concentration of buffer had no appreciable effect on enzyme activity. However, at 0.01 *M* there was slightly less activity, probably because the buffering capacity was too limiting.

TABLE II

DECREASE IN RELATIVE VISCOSITY WITH TIME DUE TO ACTION OF RHOZYME A-4 ON GELATIN[a] IN BUFFERED SOLUTIONS OF DIFFERENT MOLAR CONCENTRATIONS

Molar Concentration of Buffer	Reaction Time (Minutes)			
	5	30	60	90
	Blank η_r – Reaction Mixture η_r			
0.2	0.38	1.34	1.95	2.30
0.1	0.48	1.41	2.02	2.38
0.05	0.43	1.38	2.00	2.37
0.01	0.08	1.00	1.68	2.08

[a] After combining 5% gelatin solution with enzyme solution the final concentration was 4.55%. Enzyme concentration 1 mg. per 11 ml. reaction mixture, Temperature 40°C., pH 4.7.

B. Type of buffer. A number of different buffer solutions were used in order to hold pH constant at various pH levels. A comparison of different buffer mixtures at 0.05 *M* and the same pH was made in order to determine their effect on the proteinase activity. The results, given in Table III, show that for any given pH the type of buffer had no apparent effect on the enzymatic breakdown of the gelatin. However, McIlvaine's buffer was not considered satisfactory because the viscosity of the gelatin solutions at the lower pH values showed a gradual decrease with time on storage at approximately 4°C. The other gelatin-buffered solutions did not show this decrease.

Also presented in Table III are replicate determinations made on two different samples of gelatin. These results indicate that the gelatin viscosity method is capable of very good replication. A sample of gelatin was also obtained from a different company and the viscosity of a 5% gelatin solution (4.55% after adding enzyme solution) was 2.70 after 90 minutes' incubation time. However, the change in viscosity with time was the same as in the case of the first gelatin sample. Thus, it is important to determine the time-activity curve at constant pH

TABLE III

PROTEINASE ACTIVITY OF RHOZYME A-4 IN VARIOUS TYPES OF BUFFER SOLUTIONS USING 5% GELATIN AT 40° C.

(Enzyme solution 1 mg. per 11 ml. reaction mixture)

Mixture pH	Type of Buffer[a]		Relative Viscosity after 90 Minutes' Reaction Time
4.7	Phosphate	1	3.12
4.7	Acid phthalate	2	3.00
4.7	Acetate	3	3.04[b]
4.8	McIlvaine's	4	3.18
6.1	Phosphate	1	2.51
6.2	McIlvaine's	4	2.53
8.0	Phosphate	1	2.28
8.0	Borate	5	2.18
4.7	Acetate		2.95[b]
4.7	Acetate		3.01[b]
4.7	Acetate		3.00[c]
4.7	Acetate		3.08[c]

[a] 1, 2, and 5, Clark and Lubs buffer systems (see Ref. 3); 3, sodium acetate-acetic acid buffer; 4, phosphate-citrate buffer (see Ref. 3).
[b] Wilson's Bacteriological Gelatin; obtained January, 1954.
[c] Wilson's Bacteriological Gelatin; obtained August, 1954.

of a standard proteinase preparation for each new sample of gelatin used. A large amount of gelatin should be purchased at the start of a proteinase investigation so that a constant substrate will always be available. Dry gelatin will keep almost indefinitely.

Effect of Enzyme Concentration. Enzyme concentration curves were determined at pH 4.6 and at pH 6.9 using 0.185 *M* buffer solutions of sodium acetate-acetic acid and potassium phosphate-sodium hydroxide respectively. The results, in Fig. 3, show that a straight-line relationship exists between change in relative viscosity and increase in enzyme content for the lower enzyme concentrations in a reaction mixture which contains 4.55% gelatin. The substrate apparently becomes limiting at about 0.9 and 0.7 mg. per 11 ml. of reaction mixture at pH 4.6 and 6.9 respectively. As is shown in Fig. 3 and the following section, the proteinase from Rhozyme A-4 has much greater activity at pH 6.9 than at 4.6.

Effect of pH on Proteinase Activity. In investigating the effect of variations in pH on proteinase activity, the following buffer solutions at 0.185 *M* in 5% gelatin solution were used over the different pH ranges: A, pH 3.5 to 5.4 inclusive, sodium acetate-acetic acid; B, pH 6.0 to 7.0 inclusive, potassium phosphate-sodium hydroxide; C, pH 8.0 to 9.9 inclusive, boric acid, potassium chloride, sodium hydroxide. A number of different enzyme preparations were investigated and in

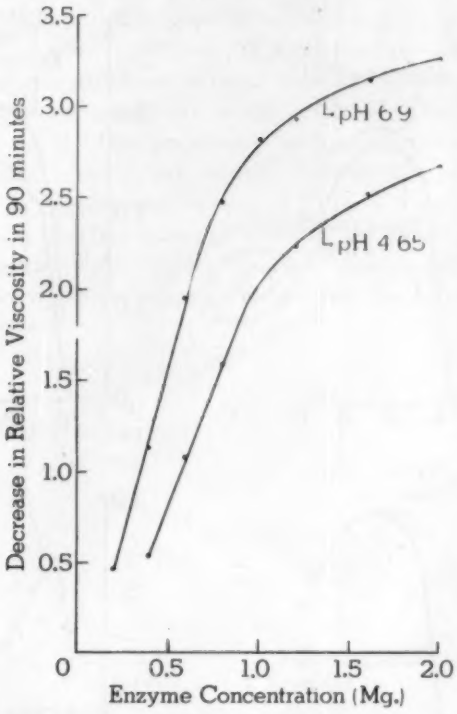

Fig. 3. Hydrolysis of gelatin at various concentrations of Rhozyme A-4. (4.55% gelatin at 40°C., mg. Rhozyme A-4 per 11 ml. reaction mixture.)

each case a sufficient quantity was extracted so that a considerable change in relative viscosity occurred at the optimum pH during a 90-minute incubation period. Results for the investigation of different fungal preparations are presented in Fig. 4 and of other products, in Fig. 5.

The pH optimum for all fungal enzymes except one was rather broad and occurred between about 6.0 and 7.5. Below pH 4.7 and above pH 8.0 the decrease in activity is very rapid for Rhozyme A-4 and Rhozyme S, and presumably also for the other concentrates from *Aspergillus oryzae*. Rhozyme P-11 had much higher proteinase activity than did the concentrates from *A. oryzae*, but the effects of pH were very similar. Figure 4 shows that in 11 ml. of reaction mixture containing 4.55% gelatin, 0.4 mg. P-11 had approximately the same activity as 1 mg. A-4. This large difference between Rhozyme A-4 and Rhozyme P-11 was not shown by the different methods used by Bowlby *et al.* (2). Their results with the hemoglobin method showed Rho-

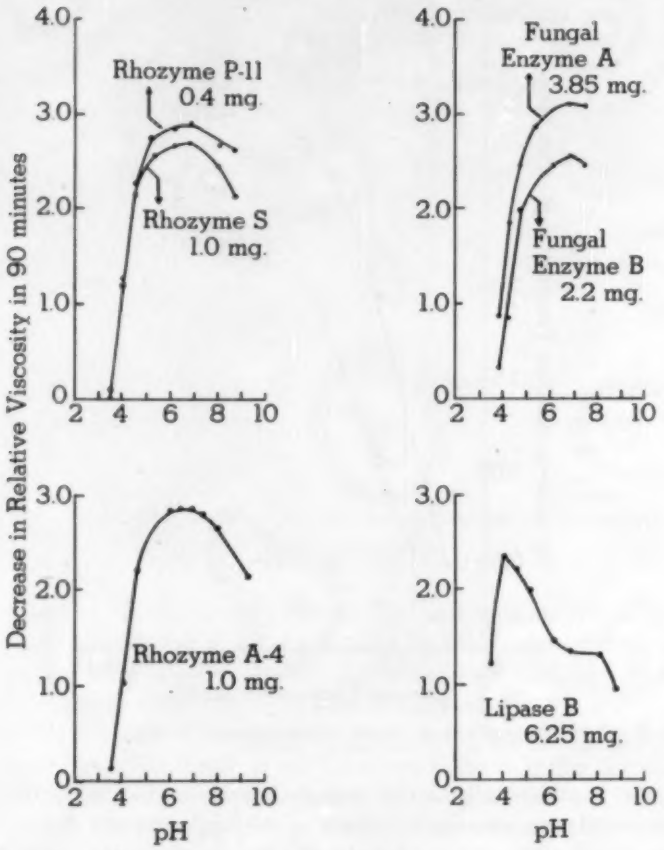

Fig. 4. Effect of pH on the proteinase activity of various fungal products. (4.55% gelatin at 40°C., enzyme product concentration per 11 ml. reaction mixture.)

zyme A-4 had greater proteinase activity than Rhozyme P-11, whereas the farinograph method showed the opposite relationship between the two products.

The results, using gelatin as a substrate for the fungal proteinases, agree quite well with results of Dworschack *et al.* (4) and Pechmann (21) concerning pH optima and broadness of the optimum range. However, when hemoglobin was used as the substrate, the pH optimum was shifted to about pH 4.1 and the peak was sharp, as illustrated in Fig. 1. These results confirm those of Miller (17) and Johnson and Miller (8).

The fungal preparation which showed a very different pH activ-

ity curve (Fig. 4) is called Lapase B.[7] It has a fairly sharp optimum at pH 4.1, decreases in activity to pH 6.2 where the curve flattens to pH 8.1, and then again shows a rapid decrease in activity above pH 8.1. This indicates the possibility of the presence of two different proteinases in Lipase B. The proteinase with an optimum at pH 4.1 shows the greatest activity, but is still considerably less active than the proteinase of *Aspergillus oryzae*. At pH 4.7 the proteinase of Rhozyme A-4 was over six times as active as the proteinase of Lipase B. The flat portion of the Lipase B curve between pH 6 and 8 corresponds to the optimum range obtained for the other fungal proteinase preparations.

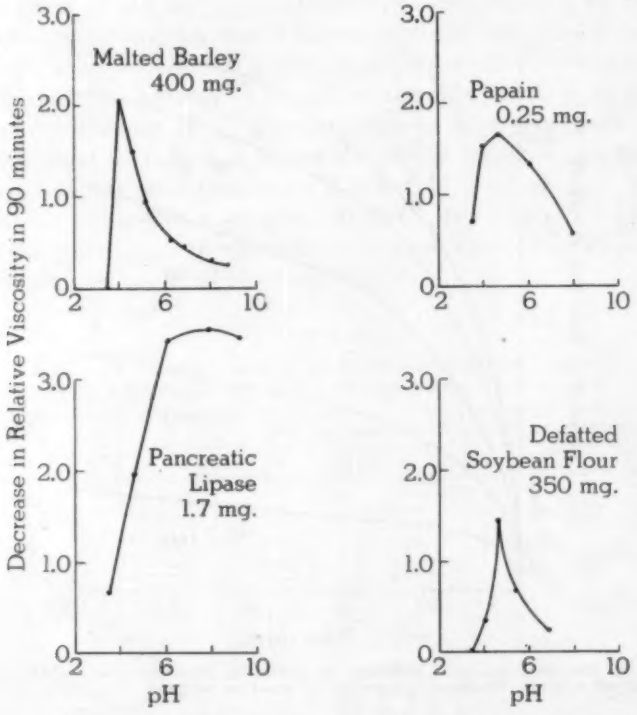

Fig. 5. Effect of pH on the proteinase activity of various plant and animal products. (4.55% gelatin at 40°C., enzyme product concentration per 11 ml. reaction mixture.)

The pH activity curves for plant and animal proteinases are presented in Fig. 5. The source of animal proteinase (pancreatic lipase) showed a pH optimum about 8.0. The plant proteinases, on the

[7] See footnote 4.

other hand, had pH optima on the acid side of neutrality. The pH optima of untreated papain, defatted soybean flour, and malted barley were approximately 4.7, 4.7, and 4.1 respectively. The pH optima for malted barley and defatted soybean flour were very sharp, showing rapid decreases in proteinase activity on either side of the optimum point. These results agree well with previous work which showed that in general the plant proteinases have their optimum activity in the slightly acid range.

Effect of Time on Change in Viscosity. The time course of the reaction of the proteinase from Rhozyme A-4 on a 4.55% gelatin solution is shown in Fig. 6. The experiment was carried out at pH 6.9 which was about in the middle of the optimum range for this pro-

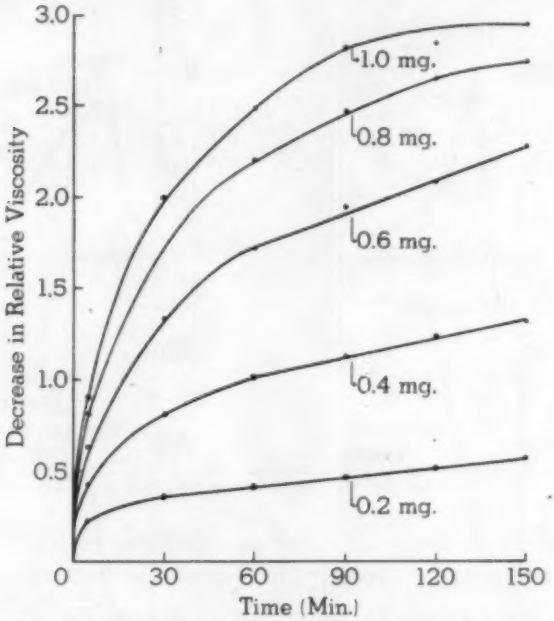

Fig. 6. The time course of digestion of gelatin by Rhozyme A-4. (4.55% gelatin at 40°C. and pH 6.9; mg. Rhozyme A-4 per 11 ml. reaction mixture.)

teinase. Various enzyme levels were used and it was found that between 60 and 150 minutes a straight-line relationship occurred for the decrease in viscosity with time up to and including 0.6 mg. Rhozyme A-4 per 11 ml. reaction mixture. At 0.8 mg. per 11 ml. and above, the decrease in viscosity was curvilinear throughout the reaction period.

Effect of Salt on Fungal Proteinase Activity. It has been stated that sodium chloride has an inhibitory effect on proteinases. Miller and Johnson (18) demonstrated that the presence of sodium chloride in the reaction mixture decreased the production of nonprotein nitrogen from hemoglobin by a fungal proteinase over 50%.

When sodium chloride was added to a gelatin solution no enzyme inhibition was evident. Since it is well known that sodium chloride has a pronounced effect on the gluten of flour and that the effect of proteolytic enzymes is greatly reduced in the presence of salt, it is believed that the decrease in proteinase activity is due to a change in the availability of the substrate rather than to enzyme inhibition *per se.*

The effect of salt on proteinase activity was measured by the gelatin viscosity method for two different levels of salt. The concentrations used were 0.4 and 1.0 g. per 22 ml. of reaction mixture which was equivalent to 4.4 and 11 g. sodium chloride per 11 g. of gelatin. These concentrations of salt per g. of protein were very much higher than that used in making a bread dough. In bread dough the concentration of salt would be about 2 g. per 11 g. of protein. The results of this investigation are given in Table IV. The relative viscosities of the mixtures with salt were somewhat lower than those without salt after 90 minutes' reaction time.

TABLE IV

EFFECT OF SODIUM CHLORIDE ON FUNGAL PROTEINASE ACTIVITY
(4.55% gelatin, pH 4.7, 40°C.; enzyme source Rhozyme A-4)

Enzyme Preparation	NaCl	Relative Viscosity at Reaction Time of (Minutes):			
		5	30	60	90
mg.	*g.*				
0	0.0	5.22	5.17	5.13	5.09
0	0.4	4.95	4.88	4.85	4.84
0	1.0	4.72	4.67	4.65	4.60
1	0.0	4.81	4.02	3.47	3.11
1	0.4	4.51	3.77	3.12	2.91
1	1.0	4.42	3.78	3.39	3.00

For the mixtures without enzyme there was a decrease of approximately 5% in relative viscosity for each increment of salt added and the percentage decrease in viscosity was found to remain fairly constant throughout the 90-minute reaction time.

When enzyme was added without salt the viscosity was lowered by 1.98 units which was a decrease of 39% in 90 minutes. In the pres-

ence of 0.4 and 1.0 g. salt, the enzyme lowered the viscosity 1.93 and 1.60 units which was a decrease of 40 and 35% respectively in 90 minutes. These results show that the rate of reduction in the viscosity of a gelatin solution by fungal proteinase was not affected by 0.4 g. salt per g. of gelatin, although there was a lowering of the viscosity by the salt alone. At a concentration of 1 g. salt per 1 g. gelatin there was a slight decrease in enzyme activity. However, this was probably due to a change in the availability of the substrate, as indicated by the lowering of the viscosity of the blank, rather than enzyme inhibition. The salt effect in bread dough may be due to the effect of salt on the gluten substrate rather than on proteinases themselves.

The gelatin viscosity procedure of Northrop for estimating the proteinase activity of various products, especially fungal proteinases, meets the requirements of a good research and control method. Reproducibility is very good, as shown in Table III, the various experiments at pH 4.7 agreeing within less than $\pm$ 3% of the average relative viscosity. The method is simple, rapid, and accurate and is conducive to handling a fairly large number of samples. As many as ten samples can be run conveniently at the same time.

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GRAIN STORAGE STUDIES. XVII. EFFECT OF MOLD GROWTH DURING TEMPORARY EXPOSURE OF WHEAT TO HIGH MOISTURE CONTENTS UPON THE DEVELOPMENT OF GERM DAMAGE AND OTHER INDICES OF DETERIORATION DURING SUBSEQUENT STORAGE[1]

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ABSTRACT

Surface-disinfected and mold-inoculated samples taken from a lot of sound hard red spring wheat were stored at room temperature and approximately 14% moisture after being held at 15, 18, and 21% moisture for 5, 10, and 15 days. The series at 21% moisture was also stored at about 13% moisture. High levels of mold infestation markedly increased the losses in viability, development of germ damage, and increases in fat acidity which occurred during temporary storage at elevated moisture contents. During subsequent storage at 14 and 13% moisture the mold counts of the majority of the samples decreased, but germ damage and fat acidity continued to develop, especially in the samples which had been heavily infested with molds. The inoculated samples temporarily exposed to 21% moisture gave large yields of flour of high ash content and poor color. Heavy mold infestation was also associated with poor baking strength and loss in baking quality during storage. Negative correlations were obtained between free fatty acids and loaf volume, germ damage and loaf volume, and logarithm of viability and free fatty acids. Losses in viability preceded the discoloration of the germ and were indicative of incipient damage and poor storage properties.

Germ-damaged or "sick" wheat is recognized by a brown discoloration of the embryo, which may be observed by removing the bran layers covering the germ. Such damage appears to be associated with high fat acidity (3, 4, 15, 19, 20, 25), low viability (3, 4, 15, 20), and often with invasion of the embryo by molds (4, 12, 19, 20, 25). The exact nature of the brown pigment in the germ is not known, but Milner, Christensen, and Geddes (19) suggested that a browning reaction of the Maillard type may be the fundamental cause of its development. Recently Cole and Milner (9) and McDonald and Milner (17) have presented experimental evidence in support of this view.

Storage of wheat at high moisture contents and temperatures is known to favor germ damage and active mold growth (3, 4, 12, 13, 19, 20, 25), but the role of microorganisms in the development of the sick wheat condition is not altogether clear.

[1] Manuscript received February 11, 1955. Contribution from the Departments of Agricultural Biochemistry and Plant Pathology-Agricultural Botany, Institute of Agriculture, University of Minnesota, St. Paul, Minnesota. Paper No. 3332 of the Scientific Journal Series. The data in this paper are taken, in part, from a thesis presented to the Graduate School of the University of Minnesota by Heinz Sorger-Domenigg in partial fulfillment of the requirements for the M.S. degree, July 1954.

Germ damage has been reported to occur in the absence of active mold growth in the seeds (3, 16, 17, 23); however, these results must be accepted with reservations, since in some cases mold growth was judged only by visual inspection of the seed and no attempt was made to determine the extent to which the seed had been invaded by molds prior to the tests.

It has been the experience of grain elevator operators that some parcels of wheat are more subject to deterioration than others, when stored at moisture contents of 13 to 14.5%. The rather wide fluctuations which can occur in the moisture content of grain in different parts of a bulk lot may account for some of the deterioration noted at supposedly low or safe moisture contents (8). However, it is also quite conceivable that the history of the grain between harvest and final storage may greatly influence its keeping qualities. Although a few investigators have observed this to be the case (18, 22), no systematic studies have been made of the importance of this factor.

The primary aim of the present study was to determine whether sound wheat exposed to high moisture contents and heavy mold invasion for relatively short periods of time would continue to deteriorate when stored at presumably safe moisture levels.

Materials and Methods

Treatment of Wheat Samples. A parcel of Marquis wheat from the dry-farming area of Montana, grading No. 1 Heavy Dark Northern Spring, with a viability of 95% and a low mold count,[2] was cleaned and divided into two lots.

The first lot (20 kg.) was divided into three subsamples, each of which was inoculated with appropriate volumes of a water suspension of mold spores calculated to give samples containing 15, 18, and 21% moisture respectively. The suspension, prepared by shaking moldy wheat in sterile water and decanting, contained spores of *Aspergillus glaucus, A. flavus, A. ochraceus, A. candidus, A. versicolor,* and *Alternaria* and *Penicillium* spp. The second lot of wheat (20 kg.) was treated in the same manner as the first, but in this case a 0.1% solution of sodium hypochlorite replaced the spore suspension. Thus, one half of the wheat was heavily contaminated with molds, while the other was almost free of them.

After a rest period of 24 hours to permit uniform moisture distribution, each sample was divided into three subsamples which were incubated in glass bottles at room temperature for 5, 10, and 15 days,

[2] Less than 1000 spores of Aspergillus glaucus per g. of wheat: 2-4% of the seeds yielded colonies of Aspergillus glaucus after surface disinfection; no other storage molds were present. These samples were not tested for bacteria.

respectively. During incubation the wheat was continuously aerated with air of relative humidity in hygroscopic equilibrium with each sample.[3] Sterile cotton plugs were used in the lines leading to the bottles to prevent contamination during the course of the test.

Immediately after incubation the wheat samples were placed on trays and air-dried at room temperature. Moldy and disinfected samples were handled in different rooms to avoid contamination. When the drying operation was completed, which usually took three days, the moisture content of the samples was adjusted to 14% by adding the required amounts of water. The wheat was then stored, with frequent mixing, in sealed containers for 4 days at 4°C. to permit the moisture distribution to become uniform.

One-fifth of each sample (440 g.) was subjected to various tests of quality, and the remainder placed in half-filled Mason jars, which were sealed and stored at room temperature for 2, 4, 8, and 12 months. At each testing time, the samples were transferred to a cold storage room (4°C.), and the analyses were performed at the earliest possible date.

The treatment of wheat at 21% moisture was repeated at a later date. As soon as the incubation was completed, the samples were dried to 13% moisture over phosphorus pentoxide under reduced pressure. This operation took 1 day. Each sample (1 kg.) was then kept in one bottle and samples (90 g.) were removed and tested after various storage times at room temperature.

All the samples stored at 14% moisture were kept in individual Mason jars. The samples stored at 13% moisture were kept in large storage containers which had to be opened whenever analyses were made, and it is likely that these samples had more oxygen and less carbon dioxide in the interstitial atmosphere than those stored at 14% moisture.

Analytical Procedures. Moisture contents were determined by the two-stage air-oven method when they were above 14%, otherwise by the one-stage air-oven method (1). Viability was determined mainly by the Minnesota State Testing Laboratory. Some samples were germinated by essentially the same technic in the Department of Agricultural Biochemistry. Wheat grade, germ damage, and, in some cases, test weight per bushel were determined by the U. S. Grain Inspection Division, Minneapolis. Mold counts were obtained as described by Christensen (7).

Lipids for fatty acid determinations were obtained by extracting

[3] For the wheats which were brought to 15% moisture, the air was bubbled through a saturated sodium acetate solution; for the samples at 18 and 21% moisture, the air was passed through 23.5 and 17.5% sulfuric acid solutions, respectively. These solutions provided relative humidities of 75, 85, and 90% (10, 24).

the freshly ground samples with absolute ethanol for 4 hours followed by extraction with diethyl ether for the same time. The extracts were combined and the solvent evaporated at reduced pressure under nitrogen, the residue was taken up in diethyl ether, dried with anhydrous sodium sulfate, filtered, and evaporated to dryness under nitrogen on a water bath maintained at 50°C. The fatty acids present in the lipids were determined according to the method of Ames and Licata (2) as modified by Hunter *et al.* (14). The lipids (0.1 g.) were dissolved in a mixture of benzene and isopropanol (1 + 1 by volume) and titrated with a 0.01 *N* solution of potassium hydroxide in isopropanol.

The results were expressed as the percentage of free fatty acids (as oleic acid) present in the lipids.

Experimental Milling and Baking Tests. After each storage period, the samples were subjected to experimental milling and baking tests using "micro" procedures. A 150-g. sample of wheat, tempered to 15% moisture, was milled on a two-stand micro mill following the procedure described by Geddes and Frisell (11); the flour yield was expressed as the percentage of flour represented in the total mill products which were recovered.

"Micro" baking tests were conducted by mixing 25 g. flour, 0.75 g. yeast, 1.25 g. sugar, and 0.5 g. salt and the appropriate amount of water in a micro mixer (National Manufacturing Co., Lincoln, Nebraska) for 2.5 minutes (21). The dough (scaled to 40 g.) was fermented at 30°C. for 95 minutes, passed through the National Dough Sheeter (rolls set at 3/16 inch), rounded and fermented for an additional 25 minutes. The dough was then passed twice through the National Sheeter with the rolls set at 3/16 and 1/8 inch respectively, and then rolled into loaf shape. The molding was completed by giving the dough piece ten turns in a National Dough Molder and placed in a low-form pan of the following dimensions: top, 7.1 × 4.6 cm.; bottom, 6.0 × 3.6 cm.; depth, 3.2 cm. The dough was proofed for 55 minutes and baked at 232°C. for 15 minutes. The loaves were measured by rapeseed displacement 45 minutes after baking and scored the following day. Baking tests were made using 0, 2, and 4 mg. of potassium bromate per 100 g. flour and the largest volumes were taken as an index of the potential baking value irrespective of the bromate level at which they were obtained.

Results

In considering the results of this study, it must be emphasized that unavoidable variations occurred in the experimental conditions. Al-

though it was possible to obtain, within close limits, the moisture contents which were selected for the prestorage treatment, it was difficult to dry the samples to exactly 14 and 13% for the storage trials. Moreover, some of the samples in the 14% moisture group showed slight increases at one time or another during the storage period. When these were observed the wheats were dried to within about 0.3% of the desired value. Two of the samples in the 13% moisture group which exceeded this value by about 0.5% were dried below 13.0% moisture within 2 to 5 days.

The samples were stored in sealed containers to prevent loss or gain of moisture due to changes in atmospheric humidity. Although some variations in oxygen and carbon dioxide tension undoubtedly occurred, it is known from other studies that the respiratory rates at moisture contents of 14% and below are low at room temperature. Moreover, other experiments in these laboratories indicate that decrease in viability, development of germ damage, and other deteriorative changes are actually lessened by lowering the oxygen tension and increasing the carbon dioxide content of the interseed atmosphere. During the storage trials at 14% moisture, the temperature varied from 24° to 29°C., the highest values being encountered during the first 3 months. In the tests at 13% moisture, the temperature ranged from 21.5° to 28°C., the maximum temperatures occurring between 6 and 8 months of storage.

Mold Count and Species Distribution. The mold counts recorded in Table I show that the prestorage treatment had a marked influence on the extent of mold contamination of the samples when they entered storage. The mold count increased with inoculation of the wheat, with increasing moisture content and with increasing length of the prestorage period.

Upon subsequent storage at 14 and 13% moisture, the mold counts decreased for those samples which showed initially high counts. Samples with low initial counts showed little or no change under similar conditions. In order of importance, the principal mold species present in the wheat before storage at 14% were: *Aspergillus glaucus, Penicillium* sp., *A. versicolor,* and *A. candidus.* During storage *A. glaucus* (the most xerophytic of the four identified fungi) gained gradually in relative importance and after 12 months' storage it was practically the only mold which was detected.

Prior to storage at 13%, *Aspergillus glaucus, Penicillium* sp., *A. ochraceus,* and *A. flavus* were present in the wheat and, in this case, storage did not significantly alter the relative distribution of the mold species in the grain. Shortly after completion of the present experi-

ment, one of us (C. C.) reported that *A. restrictus*, a slow-growing member of the *A. glaucus* group, invaded wheats stored at moisture contents of 13.5 to 15% for 16 months (6). Several samples from this experiment, which had been kept at room temperature for 16 months at 13% moisture, were tested according to Christensen (6) and found free of any contamination with *A. restrictus*.

TABLE I

INFLUENCE OF PRESTORAGE TREATMENT UPON MOLD COUNT AFTER DIFFERENT INTERVALS OF STORAGE AT 14 AND 13% MOISTURE

Prestorage Treatment			Spore Count per g. (000 omitted) after Different Months of Storage[b]				
Mold Contamination[a]	Moisture Content	Days	0	2	4	8	12
Samples Stored at 14% Moisture							
	%						
M	15	5	8	6	2	3	1
S	15	5	<1	1	1	0	1
M	18	5	36	2	10	6	8
S	18	5	2	1	10	10	<1
M	21	5	5800	720	76	85	6
S	21	5	32	11	34	30	1
M	15	10	<1	<1	<1	2	6
S	15	10	<1	<1	<1	10	4
M	18	10	1500	300	51	105	90
S	18	10	310	96	22	190	40
M	21	10	13000	418	41	15	150
S	21	10	2600	432	750	70	220
M	15	15	<1	16	8	155	52
S	15	15	<1	85	8	45	15
M	18	15	8500	500	220	310	190
S	18	15	900	225	180	560	160
M	21	15	85000	1000	1180	280	250
S	21	15	6200	575	1150	570	480
Samples Stored at 13% Moisture							
	%						
M	21	5	800	510		15	23
S	21	5	7	5		0	9
M	21	10	750	1160	...	52	37
S	21	10	50	300	...	36	57
M	21	15	1000	2000	...	36	100
S	21	15	800	1840	...	536	448

[a] M = inoculated with molds; S = surface-disinfected.
[b] Mold count of the original sample of Marquis wheat was less than 1000 spores per g.

Viability. Prestorage treatment of wheat at moisture contents of 18 and 21% for 5, 10, and 15 days prior to long-time storage at 14% moisture resulted in marked decreases in viability. The decrease was greater with increasing time at a given moisture content, with increasing moisture content, and with increasing mold count. Prestorage at 15% moisture did not affect viability (Table II).

TABLE II

EFFECT OF PRESTORAGE TREATMENT UPON CHANGES IN VIABILITY AFTER DIFFERENT INTERVALS OF STORAGE AT 14 AND 13% MOISTURE CONTENT

Prestorage Treatment			Viability after Different Months of Storage				
Mold Contamination[a]	Moisture Content	Days	0	2	4	8	12
Samples Stored at 14% Moisture							
	%						
M	15	5	96	93	95	84	71
S	15	5	95	93	96	96	93
M	18	5	81	83	86	77	62
S	18	5	94	94	96	92	91
M	21	5	86	17	14	19	10
S	21	5	93	83	87	85	80
M	15	10	93	98	97	96	92
S	15	10	97	96	99	96	97
M	18	10	49	45	46	47	43
S	18	10	88	91	90	87	75
M	21	10	15	13	15	10	6
S	21	10	79	72	68	69	60
M	15	15	96	96	94	23	0
S	15	15	96	89	86	48	26
M	18	15	24	30	33	30	25
S	18	15	76	79	70	71	54
M	21	15	6	8	8	12	6
S	21	15	48	54	52	48	42
Samples Stored at 13% Moisture							
	%						
M	21	5	42	36	..	31	34
S	21	5	93	92	..	97	94
M	21	10	15	15	..	16	17
S	21	10	80	80	..	79	78
M	21	15	10	12	..	11	10
S	21	15	57	58	..	52	56

[a] M = inoculated with molds; S = surface-disinfected.

During subsequent storage at 14.0% moisture, several of the samples decreased in viability toward the end of the storage period. This was usually associated with small increases in mold count and in moisture content as the trial progressed. In the trials at 13% moisture

TABLE III

EFFECT OF PRESTORAGE TREATMENT UPON THE DEVELOPMENT OF GERM-DAMAGED KERNELS AFTER DIFFERENT INTERVALS OF STORAGE AT 14 AND 13% MOISTURE

Prestorage Treatment			Germ-Damaged Kernels after Different Months of Storage				
Mold Contamination[a]	Moisture Content	Days	0	2	4	8	12
Samples Stored at 14% Moisture							
	%		%	%	%	%	%
M	15	5	0	1	0	0	0
S	15	5	0	0	2	0	10
M	18	5	0	0	6	0	5
S	18	5	9	0	0	4	0
M	21	5	17	63	65	73	78
S	21	5	10	0	0	2	8
M	15	10	5	0	0	0	0
S	15	10	0	0	0	0	0
M	18	10	3	17	15	36	35
S	18	10	2	4	0	0	24
M	21	10	10	75	23	80	85
S	21	10	1	2	30	4	25
M	15	15	5	0	0	0	80
S	15	15	5	0	0	1	0
M	18	15	0	33	19	34	36
S	18	15	9	1	3	6	12
M	21	15	23	80	88	50	92
S	21	15	11	3	0	26	30
Samples Stored at 13% Moisture							
	%		%	%		%	%
C[b]		0	0	0	..	0	Trace
M	21	5	5	4	..	17	40
S	21	5	3	1	..	0	Trace
M	21	10	0	46	..	30	80
S	21	10	0	10	..	0	7
M	21	15	72	83	..	15	90
S	21	15	10	0	..	7	30

[a] M = inoculated with molds; S = surface-disinfected.
[b] C = control — untreated.

the viability remained unchanged throughout the entire 12-month period.

Germ-Damaged or Sick Wheat. The grain inspector experienced considerable difficulty in classifying the kernels in these samples as

TABLE IV

INFLUENCE OF PRESTORAGE TREATMENT UPON THE DEVELOPMENT OF FREE FATTY ACIDS IN THE WHEAT LIPIDS AFTER DIFFERENT INTERVALS OF STORAGE AT 14 AND 13% MOISTURE CONTENT

Prestorage Treatment			Free Fatty Acids (as Oleic Acid) in the Lipids after Different Months of Storage				
Mold Contamination[a]	Moisture Content	Days	0	2	4	8	12
Samples Stored at 14% Moisture							
	%		%	%	%	%	%
M	15	5	9	7	6	10	12
S	15	5	8	7	9	8	10
M	18	5	7	9	9	10	12
S	18	5	7	7	7	8	11
M	21	5	14	21	22	25	29
S	21	5	7	10	10	11	17
M	15	10	7	7	7	8	9
S	15	10	6	7	8	7	9
M	18	10	10	15	15	17	19
S	18	10	8	9	9	10	12
M	21	10	22	28	33	32	34
S	21	10	9	12	13	14	15
M	15	15	6	8	8	11	13
S	15	15	5	8	9	10	12
M	18	15	15	19	19	20	22
S	18	15	9	11	11	12	14
M	21	15	..	39	41	40	41
S	21	15	11	15	15	17	18
Samples Stored at 13% Moisture							
	%		%	%		%	%
C[b]			7	7	..	12	7
M	21	5	8	10	..	17	19
S	21	5	7	7	..	14	9
M	21	10	16	24	..	33	40
S	21	10	8	9	..	15	11
M	21	15	23	32	..	41	47
S	21	15	9	12	..	18	17

[a] M = inoculated with molds; S = surface-sterilized.
[b] C = control—untreated.

normal or germ-damaged because of the rather gradual development of discoloration during the storage period. Despite several inconsistencies, the various experimental conditions caused marked differences in the extent of germ damage (Table III). During the prestorage treatment considerable germ damage resulted in the mold-inoculated samples, when they were held at 21% moisture; in most instances the extent of germ damage was considerably less in the surface-disinfected samples at comparable moisture contents and storage times.

During subsequent storage at 13 and 14% moisture, there was a gradual increase in the percentage of germ-damaged kernels in the samples which had been previously held at 18 and 21% moisture for a time. This increase was much more pronounced in the samples which had been inoculated with molds than in those which were surface-sterilized.

Fat Acidity. When the samples entered long-time storage, there was a markedly higher free fatty acid content of the lipids obtained from the mold-inoculated samples held at 21% moisture for 10 and 15 days than in those extracted from the comparable surface-disinfected wheats. Mold contamination or length of the pretreatment at 15% moisture had no influence on the development of free fatty acids; all the samples gave low values. Upon storage at 14% and 13% moisture, there was an increase in the fat acidity of most of the samples, especially those which had been inoculated with molds. These results (given in Table IV) show that the increase in fat acidity of wheat stored at

TABLE V

INFLUENCE OF SELECTED PRESTORAGE TREATMENTS ON FLOUR YIELD AND ASH CONTENT AFTER SELECTED INTERVALS OF STORAGE AT 14% MOISTURE

Prestorage Treatment			Total Flour Yield			Ash Content of Patent Flour[b]	
Mold Contamination	Moisture Content	Days	Months of Storage			Months of Storage	
			0	8	12	8	12
	%	%	%	%	%	%	%
M	15	10	74	73	74	0.50	0.52
S	15	10	75	74	74	0.48	0.52
M	21	5	75	74	75	0.52	0.54
S	21	5	75	74	75	0.52	0.51
M	21	10	76	75	76	0.56	0.57
S	21	10	76	74	74	0.51	0.52
M	21	15	77	79	78	0.65	0.65
S	21	15	76	74	74	0.50	0.52

[a] M = inoculated with molds; S = surface-disinfected.
[b] Data are expressed on a 14% moisture basis.

14 and 13% moisture is markedly accelerated by high mold invasion of the wheat prior to the time it enters terminal storage.

Flour Yield and Ash Content. With the equipment available, only small quantities of wheat could be stored and this made it necessary to carry out only micro milling and baking tests. These were confined to the samples stored at 14% moisture. Within the limits of accuracy of the micro milling test, the flour yields of the majority of the wheats did not appear to be materially influenced either by the prestorage treatment or by subsequent storage. However, the samples inoculated with molds and held at 21% moisture for 5, 10, and 15 days before storage at 14% moisture had poor milling characteristics. As shown in Table V, the yields of flour from these samples were high but the flours had high ash contents and were inferior in color.

Baking Quality. In view of the relatively large error of the micro baking test and the difficulties involved in obtaining comparable results when baking tests are conducted at intervals over a 1-year period, it is not surprising that some inconsistencies occurred. The baking data summarized in Table VI serve to illustrate the major trends which were observed.

Flours milled from wheats inoculated with molds and held at 21% moisture for 5, 10, and 15 days showed decreases in water absorption as large as 4% and yielded sticky doughs and loaves of low volume, inferior grain, and poor crumb color. Upon subsequent storage at 14%

TABLE VI

INFLUENCE OF PRESTORAGE TREATMENTS ON LOAF VOLUME AFTER SELECTED INTERVALS OF STORAGE AT 14% MOISTURE

Prestorage Treatment			Loaf Volume				Crumb Color			
Mold Contamination[a]	Moisture Content	Days	Months of Storage				Months of Storage			
			0	4	8	12	0	4	8	12
	%		cc.	cc.	cc.	cc.				
M	15	10	146	148	153	147	8	9	9	7
S	15	10	144	151	149	149	8	9	9	9
M	18	10	152	144	144	142	8	9	9	8
S	18	10	152	153	143	152	8	9	9	9
M	21	5	145	136	129	124	6	9	9	8
S	21	5	157	147	141	145	8	9	9	9
M	21	10	131	138	126	116	6	8	8	7
S	21	10	156	138	140	141	7	9	9	9
M	21	15	126	119	115	109	5	7	7	6
S	21	15	143	143	139	140	6	9	9	9

[a] M = inoculated with molds; S = surface-disinfected.

moisture, the breadmaking qualities of these samples continued to decline gradually with time.

The baking properties of flours milled from wheats similarly treated but held at 15 and 18% moisture were unaffected by the prestorage treatment and showed no prominent change upon subsequent storage at 14% moisture. Heavy invasion of the seed by storage molds apparently may result in a progressive reduction of baking quality even upon storage at 14% moisture.

Discussion

These studies clearly show that once wheats are heavily invaded by molds, they continue to deteriorate even when stored at moisture contents of 13 to 14%. The increase in fat acidity indicates that the lipase(s) elaborated by the molds can cause the gradual hydrolysis of lipids during storage at moisture contents too low for detectable growth of the fungi. Similarly, it is conceivable that during low-moisture storage the proteins and carbohydrates of the wheat were gradually hydrolyzed by the action of mold enzymes.

Cole and Milner (9) and McDonald and Milner (17) have produced evidence that the discoloration of the germ known in the grain trade as "sick wheat" is due to a reaction of the Maillard type between nitrogenous compounds and reducing sugars.

Other investigators have shown that extensive mold growth on wheat results in a marked increase in reducing sugars (19, 23) and a significant change in the nitrogenous fraction (5). Thus the action of mold enzymes during the prestorage treatment may provide precursors such as reducing sugars and partially hydrolyzed proteins which would be responsible for an increase in the rate at which the nonenzymic browning reaction takes place upon subsequent storage.

In these samples there was a rather close relation between the loss in viability, and increase in percentage of germ-damaged wheat with the increase in fat acidity and decrease in loaf volume of the flour obtainable from the wheat. Although the frequency distributions were not normal, the following simple correlation coefficients provide an index of the interrelations between these variables.

Correlation	r*
Free fatty acids in wheat lipids × loaf volume	−0.88
Germ damage × loaf volume	−0.77
Log viability × loaf volume	+0.84
Log viability × free fatty acids in wheat lipids	−0.92

*Value of r at 1% point = 0.27.

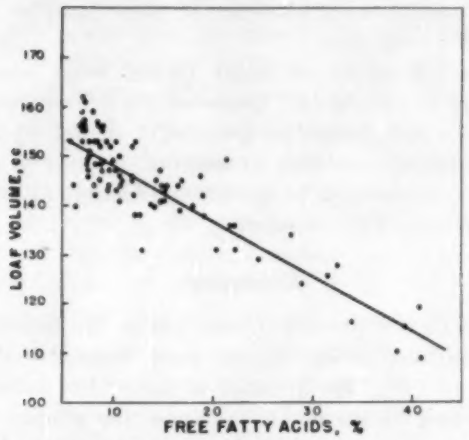

Fig. 1. Relation between loaf volume and free fatty acids in the lipids of wheat stored at 14% moisture.

The relations between these variables are shown by the scattergrams in Figs. 1 to 4.

The present technic of estimating the extent of germ damage by observing the discoloration of the germ is highly empirical. It not only fails to detect the early stages of deterioration that precede the appearance of discoloration but does not distinguish between different degrees of damage. Accurate methods for predicting the stability of grain in storage would aid greatly in avoiding losses. While not excluding the possibility that sick wheat may develop in the absence of mold growth, the present investigations provide strong evidence that

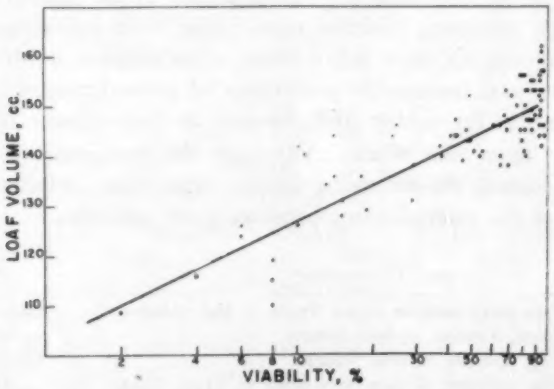

Fig. 2. Relation between loaf volume and the logarithm of viability of wheat stored at 14% moisture.

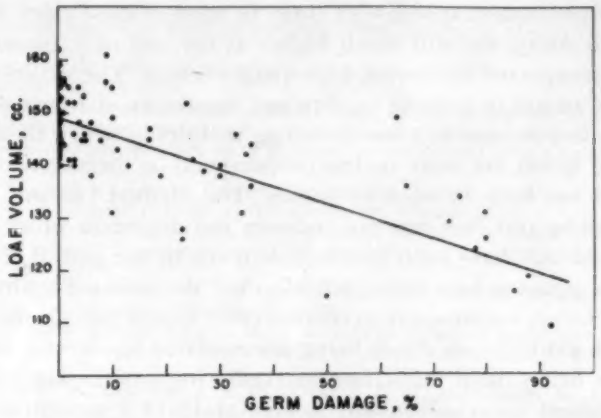

Fig. 3. Relation between loaf volume and the germ damage of wheat stored at 14% moisture.

molds are largely responsible for the losses in viability which precede the discoloration of the germ and the increases in fat acidity. The mold count of wheat has often been considered as a possible criterion of actual and incipient deterioration of wheat. However, these experiments have shown that the test has limitations as an index of deterioration for wheats stored at 14% moisture or below after they have been exposed to high moisture. Under such conditions, the mold count often decreased sharply upon storage, although other tests, such as viability, germ damage, and baking quality, indicated that extensive damage had occurred. Presumably the decrease in mold count was due to

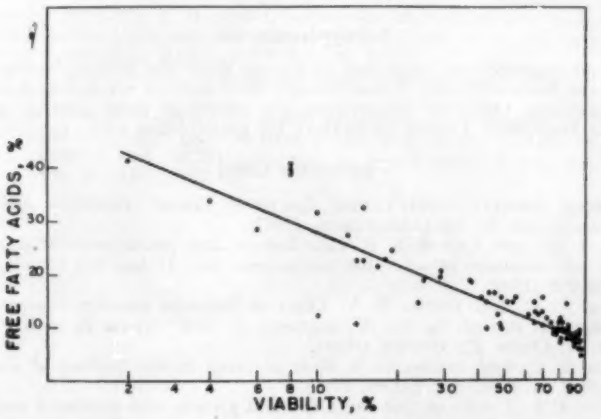

Fig. 4. Relation between free fatty acids in the lipids and viability of wheat stored at 14% moisture.

progressive death of spores with time. In most of these cases, however, the mold count was still much higher at the end of 12 months than had been reported for sound, high-quality wheat. The utility of mold count as an aid in judging quality and storability of wheat obviously depends to some extent upon obtaining samples in which the numbers of living spores are more or less proportional to the degree to which the seed has been invaded by molds. The method can not measure dead molds, and thus can not indicate the degree to which a given lot of seed may have been invaded by molds in the past, if conditions since the invasion have been such as to kill the molds. To what extent this limitation would apply to commercially stored bulks is not known, although evidence on this is being accumulated.

Since heavy mold infestation markedly impairs the keeping qualities of wheat upon subsequent storage at 13–14% moisture, and extensive mold growth is consistently accompanied by decreases in viability, low viability in itself may well be an index of poor storage stability. A combination of tests, involving moisture content, number and kinds of molds present, viability, and fat acidity should serve to predict storage behavior and extent of actual damage. The moisture content should indicate whether there is present or future danger, mold tests whether invasion of the seed has already occurred, viability whether incipient deterioration has developed, and fat acidity should give some measure of the actual damage which has already occurred. These tests all have their limitations, and improved methods of detecting and evaluating incipient deterioration of stored grain are needed before germ discoloration develops if large commercial losses are to be avoided.

Acknowledgments

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OBSERVATIONS ON THE DISTRIBUTION OF THE LINEAR FRACTION IN STARCH GRANULES[1]

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ABSTRACT

The problem of the distribution of linear molecules in starch granules is directly linked to that of the growth of the granules. In the present study, different treatments were applied to starches of normal and waxy corn, wrinkled pea, potato, and Granadilla, and the effects were studied with the microscope. Under certain conditions retrogradation phenomena could be shown to take place in waxy corn granules. Lintnerization was a suitable method to differentiate layers, even in wrinkled pea starch with high molecular association. The peculiar behavior of normal corn starch after mild treatment with hydrochloric acid made it possible to isolate blue-staining nuclei (with iodine) from waxy corn starch granules and to study the distribution of the linear fraction in the latter. In none of the starches could a gradual decrease in the proportion of linear to branched molecules from center to periphery be demonstrated. The linear fraction was either localized in the center, or regularly distributed throughout the granules.

Recently Baker and Whelan (11) suggested that in starch granules the ratio of branched to linear molecules should increase from center to periphery. In one of his last papers Meyer (24) supported this theory, stating that starch granules are surrounded by a membrane which mainly consists of molecules of the branched fraction.[2]

Meyer's statement is somewhat surprising in view of what he wrote in 1952 (23): "A special outer membrane does not exist in any kind of starch." This conclusion has been widely accepted since Alsberg (1) and Badenhuizen (3) independently demonstrated the absence of an outer membrane with special properties, and will not be discussed further in this paper.

The theory of a gradient in chemical composition, however, is new and of great interest in relation to our understanding of the growth of starch granules.

No one has ever been able to follow the growth of an individual starch granule under the microscope. Until this has been done, theories of starch granule development can be derived from indirect observations only. In the author's opinion the most probable theory is that of periodical growth by apposition (1, 3, 27). If starch granules grow this way, then it could be just as easily reasoned that peripheral

[1] Manuscript received June 4, 1954. Contribution from the Department of Botany, University of the Witwatersrand, Johannesburg, South Africa.

[2] Although the terms "amylose" and "amylopectin" are generally used for "linear fraction" and "branched fraction" respectively, in the opinion of the writer it is wrong to say, e.g., that "acid-hydrolyzed thin boiling starches have a higher percentage of amylose" (15). In order to escape such errors and for other reasons put forward by Schoch (see Patterson, 25) it is better to avoid the terms "amylose" and "amylopectin" altogether.

layers of granules are richer in linear molecules, since an increase of linear fraction has been reported for starch granules in maize and wheat kernels during their development (14, 28).

Another possibility has been indicated by MacMasters *et al.* (22). Starch granules may crystallize *in toto* from coacervates, with little or no further growth taking place once the starch has been deposited. In this case, the enzymic system in the amyloplast is supposed to build up starch molecules until conditions are such that coacervate droplets are formed. Rhythmic crystallization within each droplet leads to the formation of a lamellated spherocrystal. The theory could account for a number of phenomena which are not easily explained otherwise.

One of these phenomena is the existence of a "nucleus" which stains blue with iodine in several waxy starches. Baker's and Whelan's hypothesis is based on a study of waxy corn starch granules containing such nuclei. It is also based on the theory of apposition, as they state that "there is an exact correspondence between the order of deposition and the postulated order of synthesis."

It seems necessary to reinvestigate the problem of how a starch granule grows. As a first step, a few observations on the distribution of the linear fraction in different starches have been made in an attempt to test Baker's and Whelan's hypothesis.

Materials and Methods

Linear starch molecules, especially those of intermediate length (20), retrograde much more easily from a solution than do branched molecules. Linear molecules may form hundreds of hydrogen bonds while they become oriented in parallel fashion during crystallization. In this condition, the material may become almost insoluble in water.

In the normal type of starch granule, which contains about 25% of the linear fraction, both linear and branched molecules partake in the formation of mixed crystals (23). We can expect *a priori* that waxy starch granules, with branched molecules only, will be less resistant to the action of different agents than granules of ordinary starch. A study of the distribution of linear molecules is a study of resistance of the starch substance.

The type of resistance referred to above may be called "primary resistance." Its study should reveal natural conditions in starch granules which may vary according to percentage of linear fraction, to distribution, arrangement and chain length of molecules, or to presence or absence of fatty substances. Such factors influence degree of

molecular association, swelling power, and digestibility (5). "Primary resistance" can be studied using a method which will differentiate more-resistant and less-resistant parts, without causing appreciable swelling. The method adopted in this study was that of lintnerization (treatment of starch with 7.5% hydrochloric acid).

This treatment is just mild enough to show, for example, differences in resistance between crystalline and amorphous parts of one layer, in most starch granules, and it has the effect of exaggerating those differences. The structure of the artifacts produced is therefore based on the structure of the original starch granules. From a study of potato starch, where layers are conspicuous, we know that the lintnerized granule shows the same system and number of layers as found in the original granule. By analogy from such cases we assume the same to be true for starch granules with originally indistinct layering but which show pronounced layering after lintnerization. The chemical mechanism causing the differentiation is not important for the present purpose, and the same applies to other treatments (e.g. roasting) which cause the amorphous part of each layer to become soluble. In the latter cases, differentiation is accompanied by swelling of the residual resistant parts, which consequently separate (3).

Two other forms of resistance exist which ought to be distinguished from "primary resistance" and which were also studied:

A. "Secondary resistance," induced by the action of swelling agents which have other effects as well. Such agents are chromic acid (netting effect), sodium hydroxide (the branched fraction becomes insoluble, cf. 13), and hot water (retrogradation) (4). Under their influence, partly gelatinized starch granules will show separation of tangential layers and breaking down of those layers into separate "blocklets." These blocklets are not preformed in the granule (2). Knowledge of such phenomena is essential for the exact interpretation of starch granule structure (6).

B. "Apparent resistance." We get the impression that large starch granules of some species are less resistant than small ones, that the periphery of a starch granule is less resistant than its center part or *vice versa*. The simple mechanical explanation for these phenomena has been given earlier (3).

We find that at each stage of the normal process of gelatinization, the nearer the layer lies to the outside the less is it swollen (7). Such layers therefore can more easily undergo the influence of agents causing retrogradation, and the impression of their being more resistant than inner layers is enhanced.

It was necessary to study the influence of various swelling agents

so as to distinguish between these different types of resistance.

Treatment with Netolitzky's solution (1 g. potassium hydroxide dissolved in 9 ml. 96% alcohol), followed by addition of water, was especially used to overcome primary resistance. A granule was counted as gelatinized when it appeared to be bluish-black when viewed microscopically with phase contrast. This method was preferred to the usual one of observing the disappearance of birefringence, as it helped in eliminating confusing intermediate stages.

One sample of waxy corn starch contained a number of ordinary starch granules. This allowed for direct comparison of the waxy and nonwaxy types of granules.

Another sample consisted of waxy granules only. This and a purified sample of linear fraction were used to construct a calibration curve as required for a (admittedly rough) colorimetric determination of percentage linear fraction in starches (21).

Granadilla starch was isolated following suggestions of Cillie and Joubert (16).

Starch-formol spherites were produced by mixing one part (by weight) of starch with five parts (by volume) of commercial formol in a closed container and opening the latter after a clear gel had formed.

Results and Discussion

Primary and Secondary Resistance in Waxy and Nonwaxy Corn Starch Granules. Waxy granules showed distinct layers and blocklets under the influence of either chromic acid or 0.5% potassium hydroxide solution, and when the granules were very slowly heated in water to 70°C. (30% of the granules gelatinized). These distinct signs of retrogradation could not be found after rapid heating to 70°C. (50% of the granules gelatinized).

The appearance of layers and blocklets was exceptionally beautiful when the granules were slowly heated in 1% hydrochloric acid.

The intensity of birefringence is the same for waxy and nonwaxy granules (10). Therefore the structure of the crystalline regions should be comparable for both types. This conclusion has gained in probability since Hirst and Manners found that branched starch molecules have the shape of an elongated, rather than that of a compact, tree structure (18). It explains why retrogradation phenomena can be shown by waxy starch. In this connection it is interesting to note that starch-formol spherites with pronounced layering were just as easily formed from the branched fraction as from the linear fraction. In both types, however, molecules were found to be oriented

in the tangential, and not in the radial direction with the granule.

The molecular association in waxy starch is much weaker than in nonwaxy starch. Waxy granules are brittle structures.

It was not possible to demonstrate layers and blocklets in nonwaxy corn starch granules under the same conditions which made them appear in waxy granules, or, for example, in those of wheat starch granules. Evidently primary resistance in ordinary corn starch is so great that phenomena of secondary resistance cannot be induced. However, when ordinary corn starch is roasted (3) or subjected to ultraviolet radiation (9), separation of layers occurs in a small number of large granules after addition of water. In both cases many more granules could be induced to split off layers when they were treated with Netolitzky's reagent.

Waxy corn starch granules lintnerized for 2 weeks or longer showed pronounced layering. The residues were highly transparent, brittle, and still birefringent. Addition of iodine caused slight swelling and therefore separation of all layers, which stained yellow. Surprisingly, admixed ordinary granules assumed a blue color with iodine even after they had stayed in the acid for 1 month. Our present knowledge is insufficient to offer an explanation for this phenomenon.

Meyer (23) reported the presence of an exceptionally high molecular linear fraction in corn starch. However, all determinations of molecular weight have become obsolete since Baum and Gilbert (12) demonstrated the instability of linear starch molecules at elevated temperature in the presence of oxygen. Moreover, Foster and Paschall (17) showed it probable that not only chain length and helicoidal configuration, but also degree of aggregation influence the iodine color. This work is of special interest, as we know that the linear molecules of corn starch have a particularly great tendency to aggregation.

Starch granules of potato and wrinkled pea, which had been subjected at the same time to the same treatment, stained brown-yellow.

When iodine-stained preparations were allowed to dry in the acid medium, waxy corn starch granules dissolved and many normal ones showed separation of a number of peripheral layers after addition of water.

In the same way, in many lintnerized wrinkled-pea starch granules, containing 66% of linear fraction, a system of thin layers could first be demonstrated while the color changed from brown to blue (Fig. 1, A). Neither the action of chromic acid nor that of Netolitzky's reagent (which caused rapid swelling) showed even traces of layers in untreated granules. Evidently a further weakening of the amorphous

regions is necessary before layers, which in this case have a very low swelling power, can be differentiated.

It is concluded that layering is a general feature of starch granules. Lintnerization appears to be a method which indicates the presence and location of linear molecules in waxy corn starch.

Distribution of Linear Molecules in Waxy Corn Starch Grains. The best method found to demonstrate nuclei containing linear molecules in waxy corn starch granules was as follows: After one month of lintnerization waxy granules were washed with water, centrifuged, and dried at room temperature under a coverslip. Addition of iodine solution now produced blue nuclei standing out beautifully against a background of yellow layers (Fig. 1, B).

Nuclei could be easily isolated by adding an excess of 2 *M* calcium nitrate to the iodine-treated preparation or by drying the latter at room temperature. During these processes layers separated widely and were dissolved gradually, while the blue nuclei remained, just like admixed normal granules. It is therefore probable that the molecular framework has similar properties in both cases.

Such a nucleus could not be detected in all waxy granules. This was to be expected since Lampe (19) made the highly interesting observation that cells in the central region of the cap of the waxy maize kernel contain granules without blue-staining center spots, the latter increasing in size with distance away from this region. Here we have a problem in cell differentiation that might lend itself to detailed investigation.

The isolated nuclei had different sizes (Fig. 1, C). Some consisted only of a sphere, which in others was surrounded by one densely colored layer. In most cases, less condensed light-blue staining material was found on the outside, which suggests a decrease in concentration of linear molecules in the immediate neighborhood of the nucleus. The remainder of a granule never showed any trace of a linear fraction, and its layers all behaved in a similar manner. Whether a nucleus contained linear molecules or not, it was always birefringent when not stained with iodine. Birefringence, however, immediately became less apparent after the slightest coloration with iodine.

Therefore, if there is a gradient in the linear/branched ratio in waxy corn starch granules, it is strictly localized in the center. The small size of the nucleus adds to its resistance to swelling.

Behavior of Potato Starch Grains. Lintnerized granules, dried with iodine in the acid medium at room temperature, showed separation of layers upon addition of water. A strongly birefringent nucleus became visible, showing the same purple color as the sur-

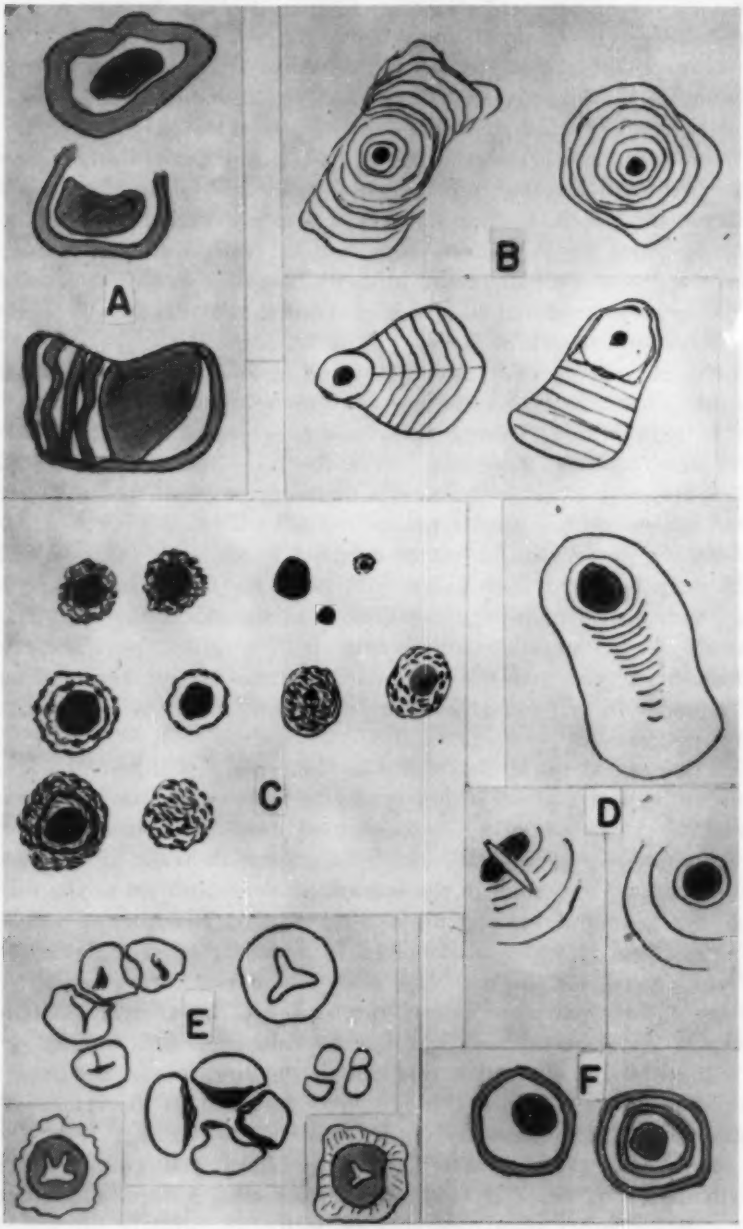

Fig. 1. Observations on the distribution of linear molecules in starch grains.

rounding layers. A blue-staining substance could be seen to diffuse into the medium from between the layers.

This treatment changed the color to blue in lintnerized wrinkled-pea starch, and to purple in lintnerized waxy corn or potato starch.

The process was repeated several times with the same potato starch granules with similar results, until after the fourth time most of the now almost colorless residues disintegrated completely. In rare cases, a nucleus became isolated, with or without one surrounding layer, and stained blue. As its swelling capacity was much less than that of the surrounding layers, it could retain a number of linear molecules, but these could be detected only after some swelling had taken place (Fig. 1, D). "Apparent" resistance accounts for these phenomena. This is in contrast to the blue nuclei of waxy-corn starch, which can be detected because of their peculiar structure (primary resistance).

When these experiments were repeated with granules washed in water, again a nucleus might become isolated here and there, but now it remained colorless.

Therefore in potato starch no indication of a gradual shift in chemical composition could be demonstrated; this is in conformity with earlier results (3). The same can be said of ordinary corn starch and wrinkled-pea starch. For those starches all observations so far point to a regular distribution of linear molecules throughout the granule.

It should now be of interest to investigate a starch containing somewhat more of the linear fraction than waxy-corn, but much less than potato starch. Such a type of starch was found in mature fruits of Granadilla *(Passiflora edulis)*.

Granadilla Starch. The properties of Granadilla starch have been described by Cillie and Joubert (16), who reported a linear fraction content of about 1%. It therefore belongs to the waxy types.

The granules in our sample stained blue first, then violet, and at last dirty brown with a darker nucleus, when iodine was added slowly. Evidently linear molecules were present throughout the granules. Whereas waxy maize starch granules stained brown immediately, those of Granadilla gave the impression of containing more linear molecules. Colorimetric measurement showed a content of 6.5% linear molecules.

Strong sulfuric acid caused iodine-treated Granadilla starch granules to stain deep blue. Deep fissures developed and divided the granules into several pieces, much in the same way as shown by wrinkled-pea starch (Fig. 1, E). Slow heating in water also produced irregular pieces and not layers and blocklets. This indicated a high

resistance, which, however, is partly "apparent," as Granadilla starch granules unfortunately are smaller than those of maize, the largest granules (which are in the minority) measuring 8–10μ. Their swelling pressure is low and therefore differentiation of layers becomes difficult. For this reason only the largest granules were investigated in more detail.

After 5 weeks of lintnerization, iodine produced a blue nucleus in all Granadilla starch granules. Sometimes this was surrounded by one blue layer, sometimes by two. In the latter case the inner layer often stained more deeply than the outer one. The surrounding layers were colorless but would become blue again after gentle heating of the preparation (Fig. 1, F).

The interesting question poses itself whether we would have obtained a picture similar to that of waxy corn starch if the granules of Granadilla starch had been of larger size. In other words: are the small starch granules of Granadilla, with 6% of linear molecules, comparable to the blue-staining nuclei of waxy corn starch, and is there a limit to the percentage of linear molecules in a starch granule, beyond which regular dispersion of the linear fraction throughout the granule occurs? Such a limit might well be at 5% (26). It is also possible that the appearance of blue-staining central spots in waxy corn starch granules is a consequence of the peculiar properties of the linear molecules of corn starch. It would be interesting to find out what the ratio of linear to branched molecules is in these nuclei.

On the whole, our results do not support the theory of a gradual decrease in content of linear fraction from center to periphery as postulated by Baker and Whelan. In most starches, linear molecules seem to be evenly distributed throughout the layers of the granules. In waxy corn starch, with only 1% of linear fraction, they are strictly localized in the center. In addition, linear molecules may be present in the surrounding one or two layers in waxy starch, but this is by no means a general picture.

We are still very far from understanding the mechanism regulating the deposition of linear and branched molecules. Although we have some knowledge of the enzymes involved, the difficulties of applying results of *in vitro* reactions to what happens in the living cell cannot be surmounted at present (8).

Acknowledgments

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RESISTANCE OF THE TESTA TO ENTRY OF WATER INTO THE WHEAT KERNEL[1]

J. J. C. HINTON

ABSTRACT

By measuring the rate at which water is absorbed from a capillary tube in contact with wheat kernels from which testa, hyaline layer, and aleurone layer were successively removed, it has been shown that the testa is the layer offering greatest resistance to water entry. No relationship was found between the permeability of the testa and color of wheat, size of kernel, grade of wheat, thickness of skin, or exposure of the wheat to adverse weathering conditions. In both hard and soft wheats, fully mealy endosperm was found to be twice as permeable as fully vitreous endosperm, but no consistent differences were found between wheats classed as hard and soft. The long resting period required in practical conditioning of wheat is associated with slow rate of water movement throughout the endosperm.

The entry of water into the wheat kernel has been studied by many investigators, their different methods of approach illuminating its different aspects. Water enters relatively quickly around the germ (9, 19) through a capillary system extending into the kernel at the point of attachment to the ear (6, 7). However, this has little practical bearing, since one hour elapses before visible water entry has occurred (20), and wheat is normally in contact with excess water for only a few minutes during the washing process. In this short time some 4 to 5% of water is absorbed by the capillary structure of the pericarp; the amount is influenced by wide variation in temperature of the washing water (5, 15, 16), degree of applied centrifugal force (12), grade of the sample (10), size and shape of kernel (5, 10, 12, 15), variety (5, 16), and previous treatment of the wheat, e.g. whether or not it has been scoured (5).

The water taken up by the pericarp is subsequently distributed throughout the kernel during the "tempering" or "mellowing" period. Inward movement takes place through the whole surface of the seed coats (20), but a barrier of low water permeability, thought to be the hyaline layer, has been shown to exist (9, 12, 20). Once within the endosperm, the rate of water movement is further influenced by temperature (12, 16), wheat type, e.g. Manitoba and soft Australian (3, 12), hardness (16), endosperm texture (3, 12), and position within the kernel (12). Recent results have shown that final equalization of water throughout the endosperm may be reached only some 60 hours after wetting (13).

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The conclusion that the hyaline layer had low permeability seemed at variance with impressions gained by the author during the dissection of wheat. A direct method has therefore been evolved for investigating the rate of movement of water through the seed coats and aleurone layer. Studies of several varieties and types of wheat, and of some factors influencing the rate of movement of water into and within the endosperm, are described in this paper.

Materials and Methods

The wheats comprised: a sample of Thatcher grown in Canada in 1947; Canadian "Standard Export Samples," 1949 crop, of grades No. 1 Manitoba Northern and No. 5 wheat; a commercial sample of Australian wheat, of soft mealy character, imported in 1951; and samples of various varieties grown in England in 1951 and 1952.

Movement of water through layers of the kernel was observed by measuring the rate of movement of the water meniscus in a capillary tube held against the surface of kernels in which different layers were exposed. All manipulations were assisted by use of a low-power binocular microscope.

Removal of individual layers (pericarp, testa, hyaline layer, and aleurone) was facilitated by moistening, which was found to have no measurable effect on rate of water absorption. In removing the pericarp, great care was exercised to avoid damaging the thin and delicate testa. The latter was removed from the hyaline layer by very light scraping while constantly maintaining a thin film of water.

Capillary tubes were 25 mm. long, 0.5 mm. outside diameter, and 0.35 mm. inside diameter. The end applied to the kernel was ground flat and slightly rounded in a flame. Tubes were calibrated by weighing the mercury contained in a measured length. The tube was supported in a tin-plate holder with a spring clip, pressing it against the kernel held in a pin vise (Fig. 1). A place was found on the kernel where the tube could be seated with no visible escape of water, and the tube was then sealed to the surface with paraffin wax dissolved in benzene. In dealing with endosperm it was essential to place the tube with a single clean movement; any manipulation lowered water absorption, presumably by forming a dough. One observation only was made on each kernel since the wax solution ran over much of the surface.

Temperature was controlled at $20° \pm 1°$C., and evaporation was prevented by introducing a second water drop into the tube near the open end. Readings of the meniscus level were taken through a microscope with micrometer eyepiece at half-hourly or hourly in-

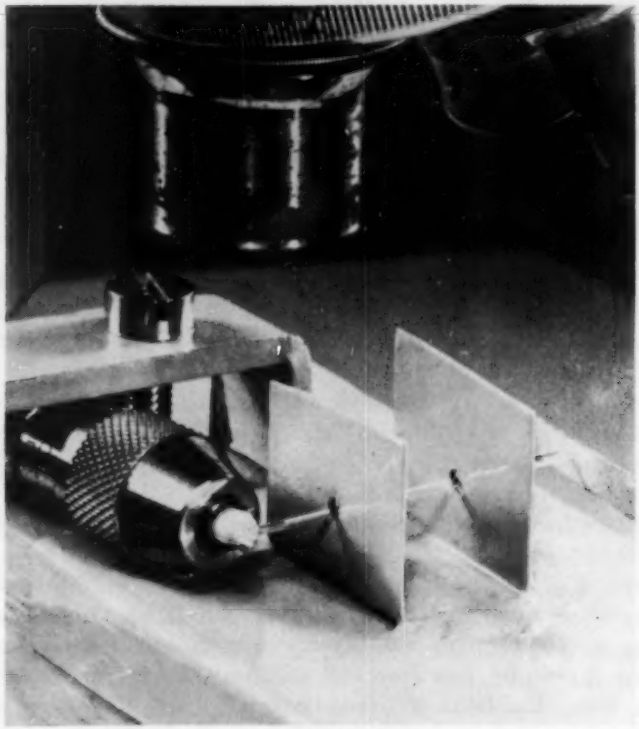

Fig. 1. Arrangement of wheat kernel and capillary tube in holder.

tervals, according to the rate of movement, for periods of 3 to 7 hours.

Rates of absorption were calculated from the movement of the meniscus and the diameter of the tube and are expressed as μl. passing through 1 sq. cm. of surface per hour. Each result given in the paper is the average of single measurements on each of 15 to 20 kernels. The probabilities of chance results are recorded with appropriate references to sections of Snedecor's book (18). Because of breakage, three tubes were used; consequently, though the figures of each section are fully comparable, they are only broadly comparable with those of other sections.

Rates of Water Movement through Different Layers

Readings for three kernels of the Australian wheat are shown in Fig. 2 to illustrate the course of water absorption when testa, hyaline layer, and endosperm are exposed by removal of outer layers. The three curves for each exposed layer represent the maximum, approxi-

mate mean, and minimum of the 15 kernels for which the mean is quoted in Table I. The curves are approximately linear. Some of the slight departures from linearity may be real (e. g. testa at 2 hours), but these have been disregarded in calculating mean rates of water movement.

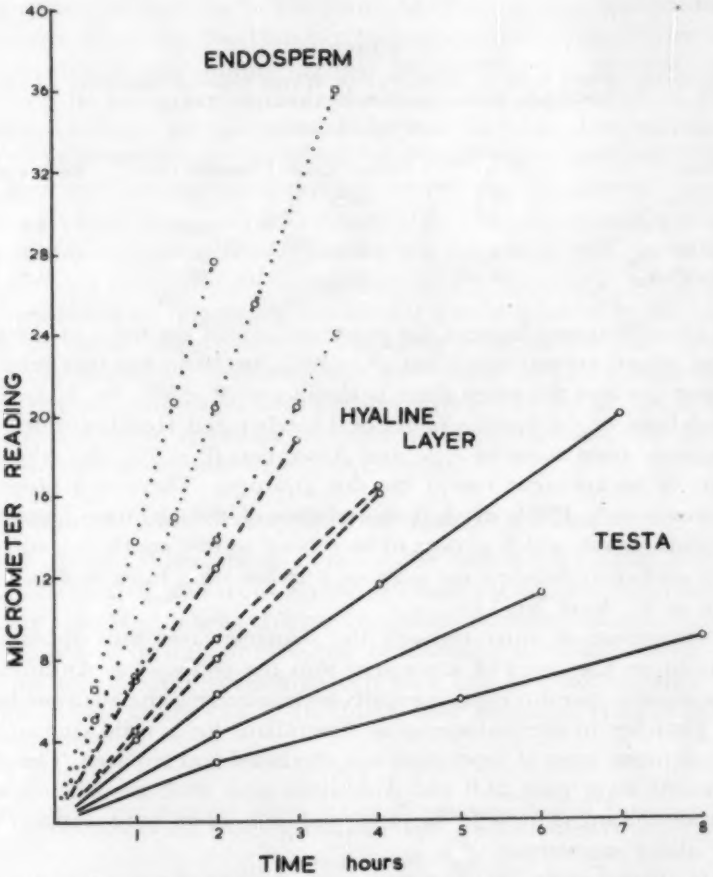

Fig. 2. Unsmoothed readings on three layers and for three kernels of Soft Australian wheat.

Table I presents mean data for four wheats on the rate of movement of water into the kernel after succeeding layers have been removed to expose the layer referred to in the headings. The testa was the outermost layer to be tested, since the pericarp layers have been shown to be very absorbent. In each wheat, the layer which effectively

limits the rate of movement is the testa, the kernel being two to three times more permeable after the testa is removed. This result conflicts with the previously held view that the barrier was the hyaline layer. This hypothesis was based on observing the movement of potassium thiocyanate rather than water (9), while subsequent observations (12) were not designed to distinguish between the hyaline layer and the testa. .

TABLE I

COMPARISON OF THE RATE OF MOVEMENT OF WATER THROUGH DIFFERENT LAYERS OF THE KERNEL OF FOUR VARIETIES OF WHEAT

(μl/sq cm/hour)

Variety	Testa	Hyaline Layer	Aleurone Layer	Endosperm
Thatcher	5.9	18.2	18.7	15.4
Atle	5.6	14.7	18.4	18.4
Holdfast	5.6	17.6	27.9	26.4
Australian	7.0	15.1	32.9	35.2

The differences between the permeabilities of the testas of the first three wheats are not significant ($P = 50\%$, Sn. 10.8); but that between Australian and the other three is significant ($P = 5\%$, Sn. 10.8). Permeabilities of the hyaline layers of Thatcher and Holdfast differ significantly from those of Atle and Australian ($P = 5\%$, Sn. 4.5), but there is no apparent reason for this grouping. There is a range of approximately 100% in the permeability of the aleurone layer and the endosperm, which appear to be related to one another; a correlation coefficient between the pairs of 0.98 has been calculated (significant at 3% level, Sn. 7.6).

Movement of water through the aleurone layer thus appears to depend on the speed of movement into the endosperm. An attempt was made to test this experimentally by transferring the aleurone layer of Thatcher to the endosperm of Australian; for a valid comparison the aleurone layer of Australian was also lifted and replaced. Thatcher aleurone layer gave 24.0 and Australian gave 26.5; the difference is not significant ($P = 20\%$, Sn. 4.5), and this offers some support for the above suggestion.

In Thatcher the speed of movement through the aleurone layer is significantly greater than that into the endosperm ($P = 1\%$, Sn. 4.5). The most probable explanation is that there is some lateral movement into the adjoining aleurone cells which, however, appears in the figures only for Thatcher. In the other three wheats the two layers are not significantly different and it is thought that this could be explained if the endosperm is less uniform in these wheats than in

Thatcher. When the aleurone measurements were made the outermost endosperm layer was effective but it was unavoidably removed in taking away the aleurone layer and preparing the surface, so that measurements for the endosperm were made at about the third cell layer inwards. Differences between the layers of the endosperm are known (8, 11, 14), and as the outermost is often of more marked vitreous texture it is likely to be less permeable than the one measured as endosperm. However, the Thatcher endosperm was very fully vitreous and apparently very uniform so that the difference in permeability may well be much less marked, providing some explanation of the difference between the results from this and the other three varieties.

The figure of 5.9 mg. per sq. cm. per hour, shown in Table I for the testa of Thatcher, was checked against the amount of water absorbed when wheat is actually soaked (12). The approximate surface area of two large kernels of Thatcher was calculated with the ellipsoid formula (17) from the dimensions of the kernels. It was then calculated that the weights of the kernels should increase by 6.4 and 6.6% per hour, respectively, by absorbing water at the rate given above. A figure of 6% weight increase per hour at 20°C. for Manitoba wheat can be interpolated from results published by Jones (12). Since many experimental errors are included in the calculations, this agreement is better than could have been expected.

Factors Affecting Water Movement through the Endosperm

The greatest difference in rates for the endosperm (Table I) lies between Thatcher, a vitreous sample of a hard variety, and Australian, a mealy sample of a soft variety. The effect of these two endosperm characters was further investigated by choosing suitable samples of vitreous and mealy kernels from two hard and two soft varieties of English grown wheats (Table II). The second sample of Holdfast had

TABLE II

COMPARISON OF THE RATE OF MOVEMENT OF WATER THROUGH THE ENDOSPERM OF VITREOUS AND MEALY KERNELS OF HARD AND SOFT WHEATS

(μl/sq cm/hour)

Class of Wheat	Variety	Vitreous Kernels	Mealy Kernels
Hard	Atle	20.2	26.8
	Holdfast (1)	20.5	26.4
	Holdfast (2)		37.5
Soft	Bersée	21.3	26.4
	Koga	20.2	36.7

a more pronounced mealy character than the first. Statistical analysis supported the impression gained from an inspection of the figures.

For vitreous kernels no significant difference was found between the varieties; it follows that there was no difference between the hard and soft wheats (Sn. 10.8). For the mealy kernels, differences between the varieties were established (P is between 1% and 5%, Sn. 10.8) and are obviously equally due to the hard wheat Holdfast No. 2 and to the soft wheat Koga. Thus again, there is no consistent difference between hard and soft wheats.

The difference between the vitreous and mealy samples of each variety was significant (P = 1%, Sn. 4.6). The difference between the two mealy samples of Holdfast lay between the 5% and 1% probability limits of confidence (Sn. 2.12).

These results therefore show that the permeability of the endosperm was affected not by the class of wheat (hard or soft), but only by whether the kernels were vitreous or mealy in character. Mealy endosperms were more permeable, the degree of permeability depending upon the degree of mealiness. This finding supports a previous observation by Jones (12). It is, at first sight, in disagreement with other reports (5, 15) for vitreous and mealy kernels, but these refer only to the initial period of absorption by the pericarp. Schäfer (16) showed that the moistening of the endosperm was slower in hard than in soft wheats, but did not record the mealiness of the samples. It is quite probable that wheats selected as hard would also be vitreous and *vice versa*.

A small but significant difference (P = 5%, Sn. 4.5) was found between the permeability of the endosperm on the back of Thatcher grain, 17.6, and that in the cheek region, 15.4. This verifies an earlier observation (12) and probably results from a difference in texture in the two regions. The change from vitreous to mealy texture during ripening occurs at the back before the cheek. The kernels tested were fully vitreous, but it is also known that vitreous endosperm may change to mealy on moistening (13), and it seems possible that this takes place more readily in the back region.

A comparison was also made between the rate of movement at the tangential surface with that at a radial surface. The application of these terms is illustrated in Fig. 3, and the results for Thatcher endosperm were: tangential surface, 17.6; and radial surface, 21.0. The difference is significant (P is less than 1%, Sn. 4.5), but the reason for this is not clear. The only structural difference between the two faces is that the tangential includes a greater area of cell wall than the radial, since the cells in the latter region are radially long and narrow;

it is thus possible to postulate a difference in permeability of regions of the cell near the wall. A more probable explanation is that two different regions of the endosperm were being measured. The diameter of the tube was about 500μ and the radial face would include endosperm to this depth; the tangential face was confined to cells at one depth in the neighborhood of 150μ. Possible differences in permeability between cells at different depths have already been discussed.

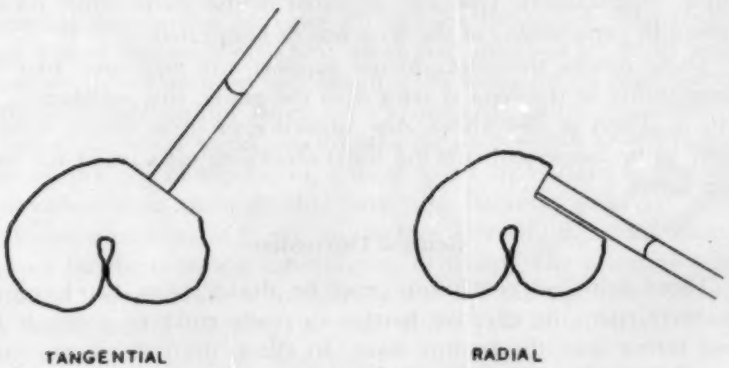

Fig. 3. Drawings illustrating tangential and radial settings of the tube.

Influence of Some Factors on Rate of Movement through the Testa. It has been shown or suggested that the following features may influence the entry of water into the kernel: uniformity of grade (10, 15); adverse weathering in the field, and apparent thickness of the skins (16). As the testa is the effective layer limiting water entry into the kernel, the influence of these factors on its permeability was investigated by comparing selected samples of wheat.

The influence of grade and size was tested by selecting the larger kernels from the No. 1 Manitoba Northern and the smaller ones from the No. 5 wheat, but rounded, well-filled kernels were always chosen. The average weights were 35 mg. and 28 mg., respectively, and the rates of water movement were 6.1 and 5.9. The difference between the two samples is not significant (P between 40% and 50%, Sn. 4.5).

The influence of adverse weathering, a feature of low-grade wheats in some years, was present to some extent in the No. 5 Manitoba but was further tested on English-grown samples. Weathering in this case means repeated wetting near harvest time, and it is apparent from the following data that it has no effect on the permeability of the testa.

	Weathered	*Not Weathered*
Bersée (thick-skinned)	5.9	6.4
Atle (thin-skinned)	6.4	5.7

Though it has been shown that the thickness of the skin is similar over a wide range of wheats (4), earlier workers have divided wheats into two broad classes with "thick" or "thin" skin, the former taking up 2% more water than the latter in the same time (15, 16). A "thick-" and a "thin-skinned" type are compared in the above table; no difference in permeability of the testa was demonstrated.

These results, therefore, do not support any suggestion that the permeability of the testa is related to the grade, size, weathering, or skin thickness of the wheat. Any influence of these factors is most likely to be connected with the water-absorbing capacity of the pericarp layers.

General Discussion

Three principal conclusions may be drawn from the foregoing results. Firstly, the effective barrier to ready entry of water is the testa rather than the hyaline layer. In effect, the two are one compound layer, but the differentiation may have a practical bearing. Since the testa is the outer layer and is thinner and more fragile, it may be more readily damaged mechanically than the stouter hyaline layer. It is possible to visualize mechanical treatments, accidental or deliberate, which may damage the testa and materially affect the rate of water movement into the kernel. These treatments would have to be more severe than heavy scouring, which increases the uptake of water only during the initial period of rapid absorption by the pericarp alone (5) and probably has little effect on the testa.

The second conclusion is that the permeability of the endosperm is influenced mainly by the degree of mealiness it has developed. Two factors may have some bearing on this relationship. Mealy kernels contain more starch and less protein than vitreous kernels (2). If there is a difference in speed or amount of water absorbed by starch and gluten, the movement of water at the surface might be affected. However, it is doubtful that the greater starch content – 5 to 10% of the total – would be sufficient to increase rate of water movement; and moreover, it has been independently observed that there is a greater speed of penetration throughout the endosperm in mealy kernels (12). A mealy appearance is probably caused by many fine air spaces associated largely with the starch grains within the cells (1). The presence of such a capillary network might materially assist the move-

ment of water and could readily be accepted as the cause of the increased permeability. This possibility is supported by the fact that much care was needed in setting up the tube with mealy endosperm; any movement causing dough formation at the surface would destroy the fine air spaces and reduce rate of water movement.

Finally, it follows from the recent observation of Jones and Campbell (13), and from the present work, that the slow water movement in the endosperm is responsible for the long period required for the complete distribution of water throughout the kernel. The observation quoted was made on wheat which had absorbed 5% of its weight of water in the pericarp and required 60 hours for its complete distribution. Calculations based on the present results show that the whole of the water could pass through the testa within an hour. Thus, though the rate of movement is three to six times faster through the endosperm than through the testa, the distances involved in the endosperm make it of prime importance in establishing the time required for the complete conditioning of wheat. The practical significance of the resistance of the testa is that it limits the water taken up by wheat during washing to that which can be absorbed by the pericarp.

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STUDIES ON WHEAT PROTEINS.
I. POLAROGRAPHIC DETERMINATION OF THE APPARENT SULFHYDRYL CONTENT OF WHEAT PROTEINS[1]

P. DE LANGE AND H. M. R. HINTZER

ABSTRACT

The apparent sulfhydryl content of gluten and the water-soluble proteins of wheat flour may be estimated polarographically. Appropriate concentrations of the protein preparations are dispersed in an ammoniacal-cobalt medium and the wave height translated into cysteine content by reference to the wave heights for standard solutions of cysteine obtained under the same conditions. The sulfhydryl content, expressed as cysteine, of gluten prepared from flours representing 13 Dutch varieties varied from 0.28 to 0.61%; the corresponding values for the soluble proteins varied from 0.46 to 2.50%.

Most methods for the quantitative estimation of protein sulfhydryl groups employ oxidizing substrates such as porphyrindin (12), potassium ferricyanide (1), *o*-iodosobenzoate (10, 19), or SH-blocking agents such as mono-iodo-acetic acid (20) and *p*-chloro-mercuribenzoate (9, 14). Benesch and Benesch (3) applied the amperometric SH-titration of mercaptans (11) to proteins.

In 1936 Brdicka (4, 5, 6) found that polarographic analysis of a protein solution in an ammoniacal-cobalt medium yielded specific current-voltage curves, characterized as "protein waves," the height of which seemed to be proportional to the concentration within a certain range. As he had obtained similar curves by the polarographic analysis of cysteine, he ascribed the protein waves to –SH groups which were susceptible to catalytic cathodic reduction. The same conclusion was reached by Tropp *et al.* (22, 22a) in studying the formation of –SH groups during the denaturation of fibrinogen.

These conclusions receive support from other observations. Proteins, such as gelatin and silk, which are poor in sulfur-containing amino acids do not show the characteristic wave. Moreover, denatured proteins yield waves which are identical with, but significantly higher than, the native proteins (24) – a change which is, according to the nitro-prusside test, paralleled by an increase in reactive –SH groups (8). Stamberg and Bailey (21) applied these findings to a study of the effect of heat-treatment of skimmilk on its –SH content.

In examining various flour extracts by the polarograph, Laitinen and Sullivan failed to detect reactive –SH groups (13). Using other

[1] Manuscript received October 20, 1953. Cereals Department of the Central Institute for Nutrition Research T. N. O., at Wageningen, The Netherlands.

technics, Myers and Working (18) and Baker *et al.* (2) were unable to find reactive –SH groups in flour extract.

Negative results were also obtained by the present authors in carrying out amperometric studies on wheat protein solutions obtained from a number of Dutch wheat varieties. However, distinct catalytic protein waves were obtained when the polarographic analysis was carried out in an ammoniacal-cobalt solution.

The present paper describes a polarographic procedure for estimating the apparent –SH content of gluten and water-soluble protein fractions prepared from wheat flour. The method was applied to these protein fractions prepared from flours representing 13 wheat varieties.

Methods

Preparation of Wheat Proteins: Gluten Protein. Gluten was washed with buffer solution (pH 6.8) from 25 g. of flour according to Dill and Alsberg (7) and dispersed in 150 ml. of 10% aqueous sodium salicylate solution by shaking at intervals for 3 days. The resulting suspension was centrifuged (3000 r.p.m.) and the supernatant liquid dialyzed against 2 l. of distilled water for 4 days, the water being changed twice daily. One-half of the precipitate inside the dialyzing membrane was again dispersed in 100 ml. of 10% sodium salicylate solution for 2 days and centrifuged, and the resulting supernatant liquid was considered as gluten solution.

Soluble Protein. Twenty grams of flour were added to 100 ml. buffer solution (pH 6.8 prepared according to Dill and Alsberg) and mechanically stirred for 1.0 hour at room temperature, after which it was centrifuged at 3000 r.p.m. The supernatant liquid contained the soluble proteins and any simpler soluble substances present in the flour.

Protein Content. Nitrogen was determined according to the micro-Kjeldahl method of Markham (15) and converted to protein using the factor 5.7.

Polarographic Procedure. Polarograms were obtained with a Radiometer Kopenhagen Type PO3e using a normal calomel electrode as anode, a mercury velocity of 2.18 mg/second and a dropping time of 3.5 seconds, and galvanometer sensitivity of 2.10^{-3} mA. The medium consisted of 1 ml. protein solution, 1.5 ml. distilled water, 0.5 ml. of 0.02% gelatin solution, 7.5 ml. of 0.2 *N* ammonium hydroxide solution (in 0.2 *N* ammonium chloride), and 7.5 ml. 0.002 *N* cobaltous-chloride solution.

A solution of 20 mg. cysteine hydrochloride (Merck) in 100 ml. distilled water was used as a reference standard.

Results

Influence of Gluten Concentration on Wave Height. The minimum wave heights obtained with dispersions of purified gluten of various concentrations are given in Table I. Polarograms for two concentrations of gluten are reproduced in Fig. 1 and standard curves obtained with cysteine monochloride solution are shown in Fig. 2.

At gluten concentrations from about 0.9 to 2.5 mg. protein/18 ml. a distinct minimum wave was observed, the height of which was re-

TABLE I

INFLUENCE OF GLUTEN CONCENTRATION ON HEIGHT OF WAVE MINIMUM

Protein[a]	Height of Minimum[b]
mg.	*mm.*
0.88	43.0
1.18	45.3
1.76	50.0
1.86	47.0
2.49	50.4
2.65	...[c]
3.80	...[c]
8.22	...[c]
9.30	...[c]
12.45	...[c]

[a] Quantity of protein (N × 5.7) present in 18 ml. of ammoniacal-cobalt medium.
[b] Values represent the height of the minimum above the cobalt level.
[c] No minimum.

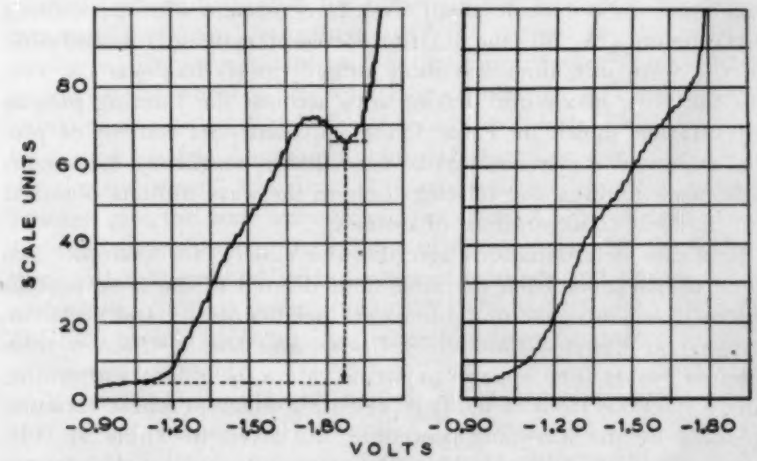

Fig. 1. Polarograms illustrating the effect of gluten concentration on wave characteristics. Curve on the left was obtained with 2 mg. of gluten and that on the right with 16 mg. of gluten present in 18 ml. of ammoniacal-cobalt medium.

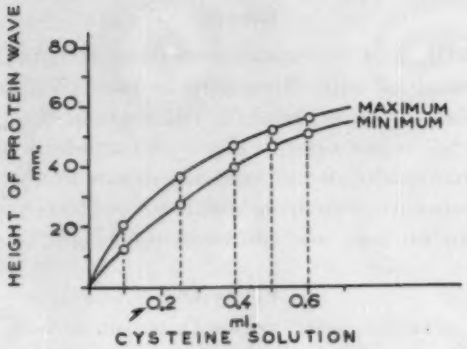

Fig. 2. Standard curve for expressing wave height of proteins in terms of cysteine. The solution contained 20 mg. cysteine hydrochloride per 100 ml.

lated to the amount of protein present. Above this range the wave leveled off to a straight line. These concentration effects are in agreement with the observations of Brdicka (4, 5, 6) and of Tropp *et al.* (23) with other proteins.

The protein waves in the ammonium-cobalt medium are similar to those yielded by cysteine solutions in the same medium. The current-voltage curve of cysteine, after a cobalt level at —0.9 volts, shows a sharply increasing current with a maximum at about —1.65 volts followed by a minimum at a potential slightly exceeding —1.8 volts. As shown in Fig. 2, there is a quantitative relation between the cysteine concentration and the height of both the maximum and minimum above the —0.9 volt cobalt level. In agreement with the findings of Wöstmann (25, 26), the relation between cysteine concentration and the wave minimum was more nearly linear than was the case with the wave maximum. Taking into account the limiting protein concentration shown in Table I, the apparent –SH content of proteins, expressed as cysteine, may be estimated by measuring the heights of the wave minima and relating these to the wave minima obtained with different concentrations of cysteine.

Replicate determinations agreed quite closely; for example, two gluten preparations from the same flour diluted to the same protein content (2 mg/ml) gave minimum wave heights of 59.5 and 60.4 mm.

Apparent Cysteine Content of Gluten and Soluble Protein from Different Flours. The apparent cysteine values for gluten and soluble protein fractions from 13 flours representing different wheat varieties, estimated by the foregoing procedure, are given in Table II. The wheats were milled on a Buhler laboratory mill and contained from 0.40 to 0.58% ash (14% moisture basis). The protein contents of the

TABLE II

APPARENT CYSTEINE CONTENT OF GLUTEN AND SOLUBLE PROTEIN FRACTIONS OF THE FLOUR FROM THIRTEEN WHEAT VARIETIES

Wheat Variety	Cysteine Calculated as Per Cent of Protein	
	Gluten Protein	Soluble Protein
Peko	0.28	0.50
Blanca	0.32	0.46
Hera	0.35	0.90
Koga	0.41	2.02
Alba	0.42	1.98
Juliana	0.56	1.18
Staring	0.61	1.94
Minister	0.55	1.48
Juliana × Kronen	0.42	2.50
Carstens V	0.48	1.92
Titan	0.28	1.82
Demeter	0.33	1.00
Bellevue	0.54	0.76

gluten and soluble protein fractions were standardized at 2 mg. and 1 mg. per ml. respectively.

The apparent cysteine content of the soluble protein fractions is higher and more variable than those of the gluten fractions. This may be partly due to sulfhydryl-containing compounds of low molecular weight in the aqueous extracts.

Discussion

No claim is made that the polarographic procedure represents a precise quantitative determination of the natural protein sulfhydryl groups which are actually present in flours. Some of the disulfide groups in the wheat proteins may be reduced to –SH groups in the ammoniacal-cobalt solution and determined as such. This is known to occur with cystine. Any nonprotein –SH groups would be included in the determination. Moreover, it is assumed that the –SH groups of proteins give the same wave height per mole of –SH as does cysteine. In order to demonstrate the validity of the method, –SH values would have to be determined by independent methods and shown to agree with those obtained polarographically. The results which have been obtained by this procedure have therefore been designated as "apparent cysteine content."

Recently, Millar (16, 17) has presented evidence which "implies that all the potential –SH groups of the protein are available polarographically." In the opinion of the present authors animal proteins in particular are denatured by the electrochemical reactions which

occur at the dropping electrode. However, the wheat proteins are more resistant to denaturation as is shown by the fact that gluten proteins can be dispersed in 10% sodium salicylate solution and recovered by dialysis with little alteration in their properties. Animal proteins are denatured by treatment with salt solutions of this concentration.

Further studies are necessary to determine whether the polarographic analysis of wheat proteins only reveals –SH groups which are available to electrochemical reduction without denaturation. The method reveals differences between the protein preparations of different flours and should prove useful as another means of studying the influence of different factors on the sulfhydryl content of the wheat proteins.

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STUDIES ON WHEAT PROTEINS.

II. SIGNIFICANCE OF SULFHYDRYL GROUPS AND DISULFIDE BONDS FOR BAKING STRENGTH[1]

P. DE LANGE AND H. M. R. HINTZER[2]

ABSTRACT

Polarographic estimations of the combined cystine and cysteine contents of the gluten and soluble proteins were made of 13 Dutch wheat varieties. When corrected for apparent cysteine contents, the cystine values of these two fractions ranged from 1.84 to 2.78% and 4.01 to 5.96%, respectively. Reproducible redox potentials, determined with a dropping mercury electrode, were obtained with each flour suspension. The addition of cysteine had a negative effect on the potential which decreased progressively as the potential approached a more negative level. The opposite effect of potassium persulfate was less pronounced although there was a tendency towards a decreasing response to its addition with increasing (less negative) level of the potential.

Baking tests and redox potential measurements made with and without additions of cysteine and potassium persulfate gave some indication that the ratio of sulfhydryl to disulfide groups in the flour proteins may be a determinative factor with regard to the improving action of potassium persulfate.

It is well recognized that the occurrence of sulfhydryl groups (–SH) belonging to the amino acid cysteine plays an important role in proteins of several biological systems. Particular importance is ascribed to the proportion in which these groups occur in relation to their oxidized form, a disulfide linkage (–S–S–), which is part of the amino acid cystine. The problem of the significance of both the reduced and the oxidized form of the –SH group in wheat proteins has been the subject of many investigations, particularly in relation to the influence of oxidizing agents on the baking strength of flours.

A clear picture of the mode of action of flour improvers has still to be established. Both Jörgensen's conception (7) based on the inhibition of proteolytic enzyme activity and the theories of Read and Haas (10), Diedering (4, 5), Wöstmann (17, 18) and others of the direct action of the protein sulfur compounds require further clarification. The same can be said of the views of several other authors, viz., Balls and Hale (1), Sullivan (13, 14, 15), and Ritter (11), who, besides postulating a direct influence on certain protein properties, allow for the possibility of an indirect action through the proteinase system.

[1] Manuscript received October 20, 1953.
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In the present investigation an approach is made to the problem by giving evidence of an existing oxidation-reduction (redox) potential in flour suspensions which may be related on one hand to the amounts of both sulfhydryl (cysteine) and disulfide (cystine) in the flour proteins, and on the other hand, to the effect of potassium persulfate on loaf volume.

Materials and Methods

The experiments were conducted on flours milled from 13 Dutch wheat varieties on a Bühler laboratory mill. The flour extraction varied from 64.4 to 73.2%; the ash contents of the flours ranged from 0.40 to 0.58% (14.0% moisture basis).

Preparation of Protein Fractions. Gluten and soluble protein fractions were prepared according to the methods described in the preceding paper (3). Dried gluten was prepared by drying at 130°C. one-half of the precipitate which resulted upon dialyzing the gluten dispersion against water. The protein contents of the various preparations were determined according to the micro Kjeldahl method of Markham (8) using a conversion factor of 5.7.

Hydrolysis of Protein Preparations. One hundred mg. of the dry purified glutens were hydrolyzed by boiling with 10 ml. of 5 *N* hydrochloric acid solution for 16 hours, filtering and making up to 50 ml. with distilled water. Hydrolysates of the soluble protein fractions were prepared by boiling 7.5 ml. with 10 ml. of 8 *N* hydrochloric acid solution for 16 hours, filtering and making up to 50 ml. with distilled water.

Flour Suspensions for Redox Measurements. For the redox determinations, 2.0 g. of wheat were suspended in a mixture of 75 ml. distilled water and 25 ml. of a phosphate buffer solution, pH 6.1 (15.310 g. of potassium dihydrogen phosphate plus 3.357 g. of disodium hydrogen phosphate in 2.0 l. of distilled water).

Protein Sulfhydryl Content. The apparent protein sulfhydryl content, expressed as cysteine, was estimated polarographically by the method described in the preceding paper (3).

Cystine Content of Protein Hydrolysates. The cystine contents of hydrolysates of gluten and soluble protein were determined by mixing 1.0 ml. with 7.5 ml. of 0.002 *N* cobaltous-chloride solution, 7.5 ml. of 0.2 *N* ammonium-hydroxide (in 0.2 *N* ammonium chloride) solution, 0.5 ml. of 0.02% gelatin solution and 1.5 ml. of distilled water. A polarogram was made before and after the addition of 0.5 ml. of standard cysteine solution (20 mg. of cysteine hydrochloride in 100 ml.

of distilled water). The amount of cysteine in the hydrolysate was calculated from the two polarograms by extrapolation as described by Wöstmann (18). In this way, a figure is obtained which represents the combined cystine and cysteine content of the hydrolysate expressed as cysteine. By subtracting from this value the apparent protein sulfhydryl expressed as cysteine in the unhydrolyzed protein, the cystine content of the protein can be calculated.

Redox Potential. The redox potentials of the flour suspensions were measured with a pH meter (type Radiometer PHM3h) employing the method developed by Wöstmann (17). As shown in Fig. 1, a dropping

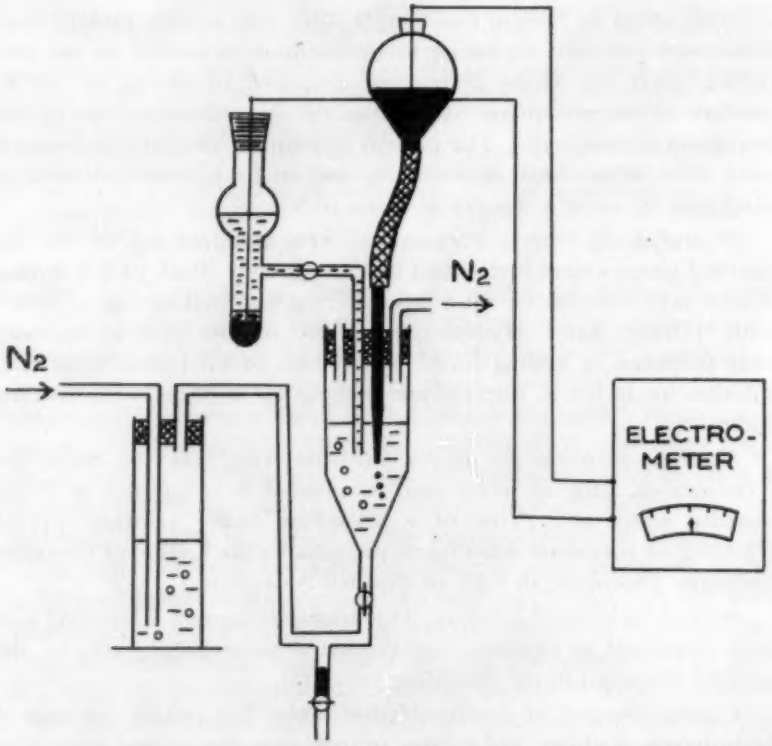

Fig. 1. Device for measurement of the redox potential of flour suspensions, according to Wöstmann (17).

mercury electrode (which serves as the cathode) and a saturated calomel electrode (anode) are connected with a millivolt measuring apparatus (electrometer). A current of nitrogen (purified and deoxygen-

ated by an alkaline pyrogallol solution) is passed through the flour suspension and the potential (E_c) is measured at 5-minute intervals. As shown in Fig. 2, a relatively constant value (A) is obtained for E_c

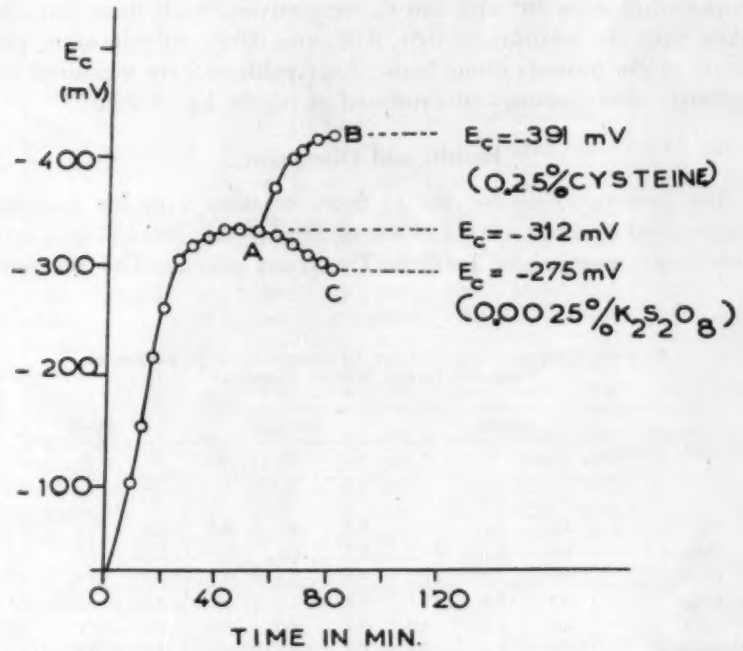

Fig. 2. Redox measurement in 2% flour suspension (pH 6). Curve AB: addition of cysteine; curve AC: addition of potassium persulfate.

in 55 to 60 minutes, and this is considered to be specific for the sample under investigation. Duplicate measurements usually agree within 2 to 3 mV.

At successive 5-minute intervals after a constant potential has been obtained, 1.0 ml. of cysteine solution (100 mg. of cysteine hydrochloride per 100 ml. of distilled water) is added and the potential is measured. In a similar manner, 1.0-ml. increments of potassium persulfate (1 mg. of the salt per 110 ml.) are added to another reaction vessel. Curve AB on Fig. 2 shows the increase of the redox potential caused by cysteine as the reducing agent; curve AC shows the decrease caused by potassium persulfate.

Baking Tests. Each flour was baked into white tin loaves according to usual Dutch practice. To 715 g. of flour were added 1.75% of yeast,

2% of salt and sufficient water to bring the dough consistency to 360 Brabender Units. Mixing time, first fermentation, second fermentation, first proof, and second proof were 8, 30, 20, 15, and about 45 minutes, respectively. Baking time was 30 minutes. Dough and oven temperatures were 30° and 250°C., respectively. Each flour was also baked with the addition of 0.01, 0.02, and 0.04% of potassium persulfate to the formula (flour basis). Loaf volumes were measured immediately after cooling and expressed as cc. per kg. of flour.

Results and Discussion

The protein values for the 13 flours together with the apparent cysteine and cystine contents of the gluten, soluble protein, and total protein are recorded in Table I. The redox potentials of the flour

TABLE I

PROTEIN, CYSTEINE, AND CYSTINE DETERMINATIONS IN FLOURS OF THIRTEEN DUTCH WHEAT VARIETIES

Wheat No. and Variety	Protein[a, b]			Cysteine[a]			Cystine[a]		
	G	S	T	G	S	T	G	S	T
	%	%	%	%	%	%	%	%	%
0 Peko	8.2	3.0	11.2	0.3	0.5	0.3	2.4	5.6	3.2
1 Blanca	8.9	2.3	11.2	0.3	0.5	0.4	2.7	4.7	3.1
2 Hera	9.0	2.5	11.6	0.4	0.9	0.5	2.8	4.8	3.2
3 Koga	9.3	2.3	11.7	0.4	2.0	0.7	1.9	4.6	2.5
10 Alba	6.9	2.1	9.0	0.4	2.0	0.8	2.3	5.0	2.9
11 Juliana	7.7	2.2	9.8	0.6	1.2	0.7	2.3	5.8	3.1
12 Staring	6.8	2.0	8.8	0.6	1.9	0.9	2.0	4.0	2.5
13 Minister	6.8	2.2	9.0	0.6	1.5	0.8	1.8	5.7	2.8
14 Juliana × Kronen	7.7	1.8	9.6	0.4	2.5	0.8	2.4	4.2	2.7
15 Carstens V	7.8	1.9	9.6	0.5	1.9	0.8	2.8	5.2	3.3
16 Titan	7.5	2.0	9.4	0.3	1.8	0.6	2.1	5.5	2.8
17 Demeter	7.3	1.7	9.0	0.3	1.0	0.5	2.5	6.0	3.3
18 Bellevue	7.7	1.9	9.6	0.5	0.8	0.6	1.9	5.2	2.6

[a] G = gluten protein; S = soluble protein; T = total protein.
[b] Protein (N × 5.7) expressed as percent dry matter basis.

suspensions and the volumes of loaves baked from the flours, with and without additions of cysteine and potassium persulfate are shown in Table II.

Theoretical Considerations. The theoretical equilibrium between cystine and cysteine is expressed by the equation:

$$R{-}S{-}S{-}R + 2\,H^+ \rightleftharpoons 2\,R{-}SH \qquad (1).$$

TABLE II

INFLUENCE OF ADDITIONS OF CYSTEINE AND POTASSIUM PERSULFATE ON THE REDOX POTENTIALS OF WHEAT FLOURS AND LOAF VOLUME

Wheat No. and Variety	Redox Potential (E_h) of Flour[a]			Volume of Loaf (in ml/kg Flour)					
	No Addition	Cysteine Added: 0.25%	Potassium Persulfate Added: 0.0025%	No Addition	Potassium Persulfate Added: 0.01%	0.02%	0.04%	Mean Increase by Potassium Persulfate	Cysteine Added: 0.001%
	mV	*mV*	*mV*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*
0 Peko	− 49	− 137	− 13	4095	4465	4680	5090	650	4185
1 Blanca	− 24	− 138	− 17	4790	5060	5130	5430	417	4490
2 Hera	− 29	− 135	− 1	5155	5605	5320	5645	368	4850
3 Koga	− 45	− 144	− 9	5145	5605	5910	5850	643	5090
10 Alba	− 66	− 145	− 29	5000	5370	5440	5430	413	4710
11 Juliana	− 56	− 146	− 28	4880	5240	5365	5500	488	4450
12 Staring	− 40	− 150	− 2	5035	5440	5375	5145	285	4940
13 Minister	− 20	− 151	− 8	5200	5280	5430	5440	183	4985
14 Juliana × Kronen	− 48	− 149	+ 1	4870	5160	5430	5205	395	4630
15 Carstens V	− 48	− 150	0	4360	4770	5090	4945	575	4310
16 Titan	− 36	− 138	+ 3	5075	5285	5250	5445	252	4860
17 Demeter	− 24	− 159	+ 17	5216	5180	5235	5385	51	4940
18 Bellevue	− 49	− 154	− 10	5375	5695	5605	5430	202	5125

[a] E_h represents the redox potential against a normal hydrogen electrode; it can be derived from E_c the redox potential against saturated calomel electrode actually measured, by the equation $E_h = E_c + 246$ mV.

According to Williams and Drissen[3] (16) this relation may be expressed by the well-known electrochemical Nernst formula:

$$E_h = E_o + RT/nF \log [RSSR]/[RSH]^2 + RT/nF \log [H^+]^2 \quad (2),$$

in which E_h expresses the redox potential against a normal hydrogen electrode of the system under investigation, and R, T, F, and n represent physical and electrochemical constants.

Upon substituting the values of these constants, equation (2) becomes:

$$E_h = E'_o + 59 \log \frac{[RSSR]}{[RSH]^2} - 59 \text{ pH} \quad (3),$$

[3] Later workers have presented evidence that the observed potentials do not arise from a simple equilibrium between the sulfhydryl and disulfide forms. Thus, Freedman and Corwin (6) demonstrated that cysteine potentials at mercury electrodes were due to an equilibrium between cysteine and a mercuric cysteinate complex. Similarly, Mauvin and Paris (9) found that following potentials with an electrode in mixtures of sulfhydryl-disulfide forms did not lead to useful results. These conclusions were based on data obtained with an immobile electrode. In the present studies, however, the measurements were made with a dropping mercury electrode. Under these conditions, cysteine-mercuric complexes would hardly be expected as the metal surface is renewed every 3 seconds. Moreover, the reproducibility of the final potentials of the flour suspensions at the dropping mercury electrode supports the assumption that mercuric complexes were not a factor.

in which E'_0 represents a specific value in mV at a definite pH for the particular redox system.

According to Borsook (2) the value for E'_0 is dependent on the method of measurement used, as is shown clearly by Table III.

TABLE III

E'_0 VALUES OF THE REDOX SYSTEM SH/SS SUMMARIZED BY BORSOOK ET AL. (2)

Measuring Method	E'_0 (pH=7)
Electrometric titration:	
With potassium iodate	+ 60 mV
With potassium dichromate	+ 130 mV
With iodine	+ 130 mV; + 270 mV[a]
With potassium persulfate	+ 115 mV[b]
Electrolytic	− 329 mV
Colorimetric	− 222 mV
Thermodynamic (calculated)	− 390 mV
Combustion	− 319 mV

[a, b] Additional data from Ryklan and Schmidt (12) and Wöstmann (17) respectively.

The present experiments gave a value of 175 mV for E'_0 by electrometric titration of cysteine solutions with potassium persulfate at pH 6.1, and this value is used in our present calculations.

TABLE IV

COMPARISON OF VALUES OF LOG [RSSR]/[RSH]^2 DERIVED FROM REDOX MEASUREMENTS (A), AND FROM POLAROGRAPHIC DETERMINATIONS FOR THE TOTAL PROTEIN (B), THE SOLUBLE PROTEIN (B1), AND THE GLUTEN PROTEIN (B2)

Wheat No. and Variety	A	B	B1	B2
0 Peko	2.38	2.55	3.37	3.06
1 Blanca	2.74	2.87	2.50	2.98
2 Hera	2.65	2.59	2.84	2.83
3 Koga	2.39	2.11	2.18	2.45
10 Alba	2.01	2.21	2.28	2.74
11 Juliana	2.19	2.30	2.77	2.48
12 Staring	2.47	2.02	2.23	2.39
13 Minister	2.81	2.19	2.55	2.44
14 Juliana × Kronen	2.34	2.12	2.05	2.75
15 Carstens V	2.34	2.25	2.37	2.68
16 Titan	2.54	2.41	2.41	2.06
17 Demeter	2.74	2.75	3.04	2.93
18 Bellevue	2.32	2.40	3.18	2.42

Correlation coefficient A–B : $+0.49 \pm 0.23$
Correlation coefficient A–B1: $+0.28 \pm 0.28$
Correlation coefficient A–B2: $+0.16 \pm 0.29$

Equation (3), rearranged, becomes:

$$\log \frac{[RSSR]}{[RSH]^2} = \frac{E_h - E'_o}{59} + pH \qquad (4).$$

The value of log $[RSSR]/[RSH]^2$ may be established in two ways, viz.: (a) from the measured redox potential E_h (value A of Table IV); and (b) by calculation from the molar concentrations of cystine and cysteine derived from the polarographic measurements. In this connection, the values of log $[RSSR]/[RSH]^2$ for each wheat variety have been separately calculated for the total, soluble, and gluten protein (values B, B1, and B2, respectively, of Table IV).

The data derived from the redox determinations (A) are, within certain limits, comparable with those calculated from the polarographic measurements for the total protein (B). The correlation between the A and B values approaches significance which suggests that there may be a relationship. On the other hand, the correlation of A with B1 and with B2 (concerning the soluble and gluten protein, respectively) is low. Taking into account the rather small number of samples analyzed and the possible experimental errors, the statement seems justified that the redox potential existing in a flour suspension may be related to the equilibrium between protein-sulfhydryl and disulfide in which both the soluble as well as the gluten proteins take part.

The effects of cysteine as a reducing agent and potassium persulfate as an oxidizing agent on the level of the redox potential are

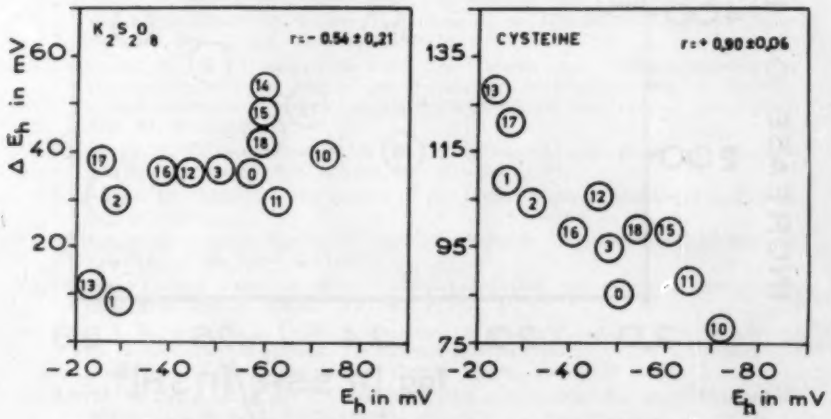

Fig. 3. Alteration of E_h of a flour suspension induced by 0.0025% of potassium persulfate (left) and by 0.25% of cysteine-HCl (right). The figures in the circles refer to the wheat varieties.

shown by Fig. 3. It appears that a striking positive correlation exists between the value of the potential (E_h) and the alteration of the potential by addition of cysteine (E_h). This means that the negative effect of cysteine on the potential decreases progressively as the latter approaches a lower (more negative) level. The opposite effect of potassium persulfate is less pronounced although there is a tendency towards a decreasing response to its addition with increasing (less negative) level of the potential.

The question whether the level of the potential values of the flour suspensions is of any importance with regard to the effect of potassium persulfate on the baking strength has also been examined. To eliminate possible errors due to discrepancies in the baking tests, the log $[RSSR]/[RSH]^2$ values (which at a definite pH are directly proportional to the redox values; see Table IV) have been compared with the mean increases of loaf volume as a result of adding three dif-

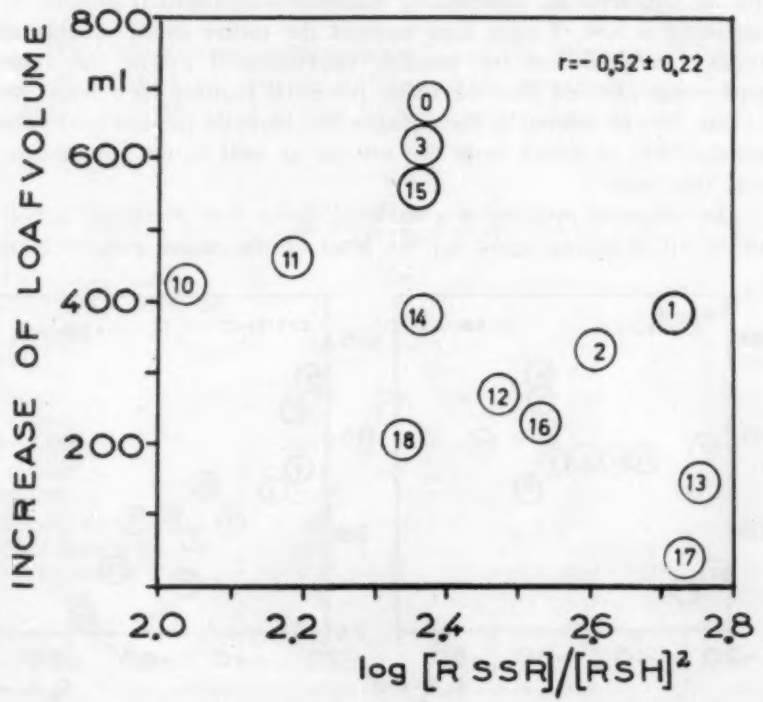

Fig. 4. Relation between redox value (expressed as log $[RSSR]/[RSH]^2$) and change of loaf volume by addition of potassium persulfate. The figures in the circles refer to the wheat varieties.

ferent concentrations of persulfate. The relevant data have been summarized in Table II, while Fig. 4 shows graphically the relationship between the mean increases of loaf volume and the log $[RSSR]/[RSH]^2$ values.

Figure 4 indicates the existence of a negative correlation between loaf volume increase and log $[RSSR]/[RSH]^2$ values. This means that a lower level of the redox potential is in some way connected with an increased susceptibility to an improving action by persulfate.

That the relationship is incomplete is not surprising; on the one hand, many factors besides those already discussed may influence loaf volume. Thus, there is a large influence of the water absorption of dough, while the numerous enzymatic processes occurring during fermentation also exert a definite effect on loaf volume. On the other hand, further work is necessary to ascertain to what extent the methods used in this study actually measure the sulfhydryl-disulfide system. There is a possibility, of course, that the net effect of a more complex system is involved. The answers to these questions must be left for future research.

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ON THE PHYSICAL BASIS OF THE RESPONSE OF ENDOSPERM DENSITY TO CHANGE IN MOISTURE CONTENT.

I. DENSITIES OF DISSECTED AND OF MILLED ENDOSPERM PARTICLES[1]

J. D. Campbell and C. R. Jones

ABSTRACT

Endosperm particles, dissected from vitreous Manitoba grains, fall in density on slight moistening, the rate of fall with increase in moisture content being indicative of contraction in the combined volume of endosperm plus water. Passage of the wheat between break rolls disturbs the structure of the endosperm even in the coarsest fragments produced (semolina or sizings), causing them to become more or less mealy. This disturbance increases the rate of fall in density on subsequent slight moistening, though it does not affect the density of the endosperm at about 12% moisture content. When the moisture content of endosperm is raised above 20% marked swelling occurs, irrespectively of whether or not the endosperm has been milled.

Mealy endosperm particles behave abnormally on drying below 12%. Their density decreases rapidly, whereas vitreous particles show a density increase, the rate of which accords with the slope of the line established for higher moisture contents.

In milling Manitoba wheat, flour produced in the early breaks is derived mainly from the softer grains present in the grist, rather than from the softer portions of every grain.

In a previous paper (2) the relationship between moisture content and density of individual particles of endosperm dissected from vitreous wheat grains was described. The present paper deals with the corresponding relationship for particles of endosperm in bulk as semolina (sizings or coarse middlings, in American terminology) and offers an explanation of apparent anomalies, some implications of which are potentially of milling interest.

Materials and Methods

Wheat and Semolina. The semolina (sizings or coarse middlings) was prepared, free from loose bran, by passing No. 1 Manitoba Northern Wheat, cold conditioned to 16% moisture content, through the first three breaks of the laboratory milling system. Fuller details of the milling system, of the wheat ("Manitoba A") used, and of the procedure for adjusting the moisture content of semolina and of individual particles were given previously (2). The initial moisture content of

[1] Manuscript received February 16, 1955. Contribution from the Research Association of British Flour Millers, St. Albans, England.

the semolina was 15%. Moisture contents were determined as previously described (2). All moisture values are expressed on the moist basis.

Moisture values for semolina (in bulk) were obtained by oven-drying weighed samples. Those for individual particles were based either on oven-drying of the well-settled grain from which the particles were dissected, or alternatively, on attainment of moisture equilibrium with semolina samples previously oven-tested and weighed. Figure 3 in the previous paper (2) afforded evidence that comparable results for moisture content of individual particles were obtained by these two methods.

Density Determination. Two quite different methods are involved: (a) a micromethod, by suspension of individual particles, (b) the usual macromethod—by displacement, ascertained by weighing.

(a) The procedure, including dissection of individual particles from wheat grains under oil and their suspension in mixtures of carbon tetrachloride and cyclohexane, was fully described in the previous paper (2).

Prior to taking individual particles from semolina for density determination by suspension, a small subsample of the semolina was covered with oil to prevent loss of moisture during the examination.

(b) Determinations of displacement of xylene by samples of semolina were made by means of a Weld specific gravity bottle (4) of approximately 25-ml. capacity. The procedure was generally on the lines described for whole grain by Sharp (3) and adopted by subsequent workers. The bottle, containing the accurately weighed quantity (about 4–8 g.) of semolina, was partly filled with xylene and shaken repeatedly during evacuation prior to "topping-up" at 25°C. A series of six replicate determinations of density on subsamples of semolina showed a standard deviation of 0.00016. All results refer to the density of water at 25°C. and to weighings in air. Strictly, therefore, the values obtained are specific gravities: for convenience they are termed densities in this paper. Their adjustment to other standards, if required, is described in the previous paper (2). The xylene became colored on contact with the semolina; special experiments showed, however, that any associated change in density of the xylene was too small to be detected by the present procedure.

To obtain assurance of comparability between the two methods of density determination, several particles, picked at random from the semolina sample, were placed in a water-saturated mixture of carbon tetrachloride and cyclohexane. The proportions of the constituents of this mixture were then adjusted to the mean point of suspension, i.e., to the point at which most particles were suspended

and, of the others, approximately equal numbers were rising and sinking respectively. Determination of the density of the liquid, by means of a pycnometer, gave a result in close agreement with that obtained by weighing a sample of the semolina, at the same moisture content, under xylene in the specific gravity bottle. Similar results were obtained when the semolina particles were covered with oil prior to transference to the liquid mixture.

Results

In Fig. 1, line A is reproduced (without experimental points) from Fig. 3 of the previous paper (2); it shows the relation between density and moisture content of particles dissected from vitreous Manitoba grains. The new line, B, applies to samples of semolina milled from the bulk of the same wheat. Values for particles of different sizes within the range studied (0.36 – 0.62 mm.) were fitted equally well by this line.

Lines A and B are of particular interest in respect to the differences in slope between their straight portions. These differences require consideration of structural changes in the endosperm which are conveniently discussed before dealing with the trends shown by lines A and B at high and low moisture contents since they affect these trends also.

Differences in Slope between the Straight Portions of Lines A and B. The straight portions of the lines (between 12 and 18 or 19% moisture content) have gradients corresponding to the following changes in density per 1% difference in moisture content: A, 0.0041; B, 0.0054. Semolina particles are derived from various regions of the endosperm, whereas the dissected particles of line A originated from the cheek centers only. Particles dissected from other regions of the endosperm, however, have been found to give lines closely parallel to A and the change in slope shown by line B can only be attributed to some physical modification sustained by the endosperm during the passage of the grain between the break rolls. This is supported by the results of an experiment in which a sample of Manitoba A, conditioned to 18% moisture content, was gently ground in a laboratory hand mill having burred disks (as in a coffee grinder). Individual endosperm particles, visually similar to those dissected from grains of the same wheat, were selected under magnification from the ground material. They were then adjusted in moisture content, by vapor equilibration, to three different levels, the lowest of which (17.9%) was the average moisture content of the ground material from the coffee mill. The corresponding density values,

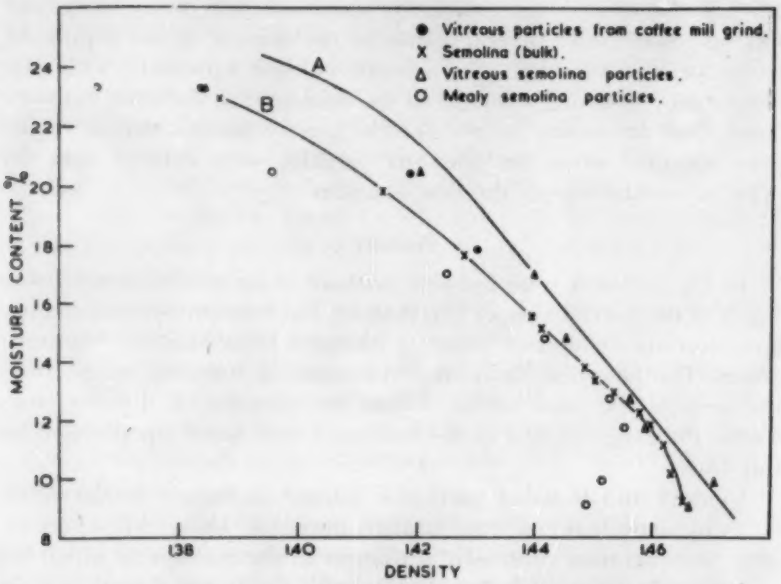

Fig. 1. The relationship between density and moisture content for endosperm particles dissected from vitreous Manitoba wheat grains (line A), and for semolina (line B) milled from the same wheat. The semolina has been tested in bulk and also in the form of individual particles.

shown by the solid circles in Fig. 1, are lower than those for dissected particles at the respective moisture contents by the amounts shown in row 1 of the following table: row 2 shows the density intervals between lines A and B at corresponding moisture contents.

	Moisture Content (%):		
Density Deficit in:	17.9	20.4	23.4
1. Coffee-mill grind	0.0064	0.0092	0.0144
2. Semolina	0.0090	0.0150	0.0250

The mean crushing stress applied to the endosperm was undoubtedly less severe during the operation of the hand-grinder than in the course of the break rolling that produced the semolina. Evidently, the degree of structural disturbance during milling affects the density fall per unit rise in moisture content. Support is given to this suggestion by consideration of the values for individual vitreous and mealy particles of semolina, represented respectively in Fig. 1 by the triangles and hollow circles. Thus, with increasing moisture content, values for the vitreous semolina particles fall mainly between lines A and B, while those for mealy particles fall more or

less far to the left. The structural disturbance has, however, little effect on the density of the material at relatively low moisture contents, 10–13%, since in this region lines A and B tend to converge.

Development of Mealiness during Breaking. In a break-roll grind, even of all-Manitoba wheat, the endosperm particles are not uniformly loosened in texture. In some, the loosening is characterized by marked mealiness; in others it is not apparent visually, even under magnification, though it may be revealed through the effect now under discussion. The results of an attempt to follow visually the development of this mealiness are shown in Table I. The particles in

TABLE I

VISUAL CHANGE IN TEXTURE OF ENDOSPERM ON BREAKING

Material	Numerical Proportion		
	Wholly Vitreous	Partly Vitreous	Mealy
	%	%	%
A: Semolina particles	23	65	12
B: Wheat grains	80	11	9
C: Wheat, excluding mealy grains	88	12	0
	Density[a] of Central Check Endosperm		
D: Six grains corresponding to row B: mean values	1.4582	1.4542	1.4368

[a] At 12.6% moisture content.

a subsample of the semolina were subdivided, under low-power magnification, into three groups: wholly vitreous, partly vitreous, and mealy; the results are shown, on a percentage basis, under the respective headings, in row A of Table I. Furthermore, 500 wheat grains of Manitoba A were bisected transversely and classified in the same way, according to degree of vitreousness of their endosperm, as in row B. Of these, 9% had entirely mealy endosperm. As will be shown, this is probably shattered so finely by the break rolls that it is not represented among the semolina particles produced by them. The effect of the breaking on endosperm texture is therefore more adequately conveyed by comparing with the results for semolina particles (row A) those for wheat grains expressed (as in row C) as a percentage of the total number less the 9% of mealy grains.

The weighted average[2] of all the density figures in row D of Table I is 1.4558 (I), while that for the first two figures only (i.e., excluding

[2] Obtained by averaging the specific volumes (i.e. the reciprocals of the densities). When, as in the present case, the differences between the values averaged are relatively small, the result of the calculation is almost identical with that obtained simply by averaging the densities.

the figure for mealy grains) is 1.4577 (II). From line B (Fig. 1) the density of the semolina at 12.6% moisture content is 1.4557. I and II, however, relate to central cheek endosperm, and for comparison with this value they need adjustment by an allowance for the contribution of endosperm from other regions of the grain. Such an allowance may be derived approximately from the data in Fig. 2 of the previous paper (2) by drawing contours for appropriate density intervals and calculating weighted averages for density on the basis of the respective areas enclosed by the contours. The result of this calculation was 0.0030. That is to say, the average density of the endosperm throughout this middle cross-section of the grain is lower than the density of the central cheek by 0.0030. The density of the endosperm in longitudinal sections was previously found to be uniform at corresponding points in the cross-section over two-thirds of the distance from the germ end, but it fell by about 0.005 towards the beard end (2, p. 186). Owing to the tapered shape of the grain the effect of the fall along the third of the length towards the beard end is relatively small; it was calculated approximately on the basis of measurements of areas of a series of cross sections at regular intervals throughout the length of the grain. The result of the calculation indicated that the adjustment already mentioned should be increased by 0.0007. That is to say, the average density of the total endosperm in the grain is lower than the density of the central cheek in the middle cross section by 0.0037. After this allowance has been applied, the figures for comparison are: I, 1.4521; II, 1.4540; semolina, 1.4557.

Unfortunately it is not possible to examine the significance of these differences statistically, because the comparison of the values for I and II with that for the semolina depends upon the validity of the allowance of 0.0037 referred to above. This in turn depends upon the contour drawing and the various area measurements, and the available data were insufficient to establish its accuracy. The standard error of the mean value, 1.4582, for density of endosperm from cheek centers of six vitreous grains, given in row D of Table I, was, however, only 0.0003, and that of the mean density for semolina (1.4557) only 0.0002. Moreover, it is reasonable that, after the allowance has been applied, the value for II should be somewhat lower than that for the semolina, for this implies that some of the endosperm from the regions of lowest density within each grain is not included in the semolina. This is to be expected, since a) it is the outer endosperm that is low in density (2, Fig. 2), and b) material from the fourth break was not included in the sample of semolina; i.e., a considerable

proportion of endosperm remained adhering to the bran. Furthermore, the uncertainty of contour drawing and determinations of areas and densities could not conceivably have been sufficient to allow the final value for I (inclusive of the mealy grains) to approximate that for the semolina.

The indication is, therefore, that the endosperm from the mealy grains cannot be present in the semolina. The further implication—that it must have been converted into middlings and break flour—is increased in interest by consideration of the relatively small yields of these materials under normal milling conditions. A traditional view is that, in the milling of an individual type of hard wheat, the flour from the early breaks is drawn from the softer, more "starchy" portions of every grain in the grist. The results now given suggest rather that it is derived mainly from the softer grains present in the wheat and that it may even represent the major part of the flour contributed by such grains.

Dry-Basis Densities in Relation to Lines A and B. The following table shows water-free densities calculated from values read from Fig. 1 for various materials at 17 and 13% moisture contents (corresponding to the limits of the straight portion of line B):

Moisture Content	*Dry-Matter Densities Calculated for:* Dissected Particles[a]	Semolina[b]	Mealy Semolina Particles[c]
%			
17	1.5826	1.5710	1.5609
13	1.5628	1.5594	1.5558
Difference	0.0198	0.0116	0.0051

[a] Based on line A.
[b] Based on line B.
[c] Based on points shown as hollow circles in Fig. 1.

The basis of the calculation[3] involved the assumption of additivity of specific volumes of water and dry substance: in other words, the assumption that the addition of moisture to the endosperm would cause an increase in volume of the system (endosperm plus water) equal in extent to the volume normally occupied by the weight of water in question. If this were the case the calculated dry-basis densities

[3] The densities of endosperm and water are so far apart, in relation to the number of significant decimal figures under consideration, that weighted averages must be based on the specific volumes, i.e. the reciprocals of the densities, and not directly on the densities. The calculation involves the following formula:

$$D = d \frac{100 - m}{100} \Big/ 1 - d \frac{m}{100}$$

where D is the dry-basis density, d is the moist-basis density, m is the percentage moisture content, and all density values are on the 25°/25° basis.

would not change as the moisture content increased. The values obtained from line A show, however, a considerable rise; over the total moisture range (9–19%) covered by the straight part of line A the dry-basis densities were found to increase from 1.5447 to 1.5930. Evidently, in unmilled grain, the combination of water with vitreous endosperm causes contraction in the total volume of the system; it is known (1, p. 585) that this may occur with colloidal materials. When, however, the structure has been disturbed through milling its capacity to share in such contraction is reduced. The values in the lowest row of the above table, which serve as a measure of this capacity, suggest that it is reduced progressively as the degree of disturbance through milling increases. The calculated dry-basis values for the mealy semolina particles are not far from constancy over the given range of moisture content.

Changes in Slope of the Lines at High Moisture Contents. Mealy semolina particles show an accelerated fall in density with increasing moisture content at an earlier stage (about 16%) than the vitreous. In Fig. 1 the points for the vitreous semolina particles fall rather irregularly, presumably as a result of variation in intensity of the milling effect sustained (in contrast, the trend of the points for dissected particles is very regular). Their positions, however, do not indicate marked acceleration in rate of density fall until the moisture content exceeds 18–19%. Above 19–20% line A, for dissected particles, bends over markedly, and comparison of the upper parts of lines A and B suggests that above 20% moisture content endosperm swells markedly, irrespectively of whether or not it has been milled.

The Trend at Low Moisture Contents. On drying below 12% moisture content, particles dissected from grain at 12.6% give values falling on an extension of the straight part of line A. From the few experimental points available, particles of vitreous semolina (whose moisture content prior to drying was 15%) appear to behave like dissected particles. On the other hand, mealy semolina particles, initially at the same moisture content, show rapidly decreasing density on drying below 12%. It is natural that line B should show some degree of curvature below 12%, with convexity towards line A, since the values on which it is based refer to a mixture of vitreous and mealy particles.

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ON THE PHYSICAL BASIS OF THE RESPONSE OF ENDOSPERM DENSITY TO CHANGE IN MOISTURE CONTENT.

II. REVERSIBILITY OF ENDOSPERM DENSITY ON DRYING IN RELATION TO BEHAVIOR OF WHOLE-WHEAT GRAINS[1]

J. D. CAMPBELL AND C. R. JONES

ABSTRACT

The fall in density of endosperm particles caused by slight moistening is fully reversible on subsequent drying, but full reversibility disappears if the moisture content has been raised to over 20%. At this level, density fall accelerates and development of mealiness is intensified.

In the case of whole grains, even slight moistening causes a density fall that is not fully reversible on subsequent drying. This difference in behavior between whole grain and endosperm must be due to changes in bran structure: endosperm modification contributes substantially to the lack of reversibility in whole grain only when its moisture content is raised above 20%.

Moistening of vitreous Manitoba grains causes partial development of relatively widely spaced endosperm cracks (which are distinct from coarse radial cracks produced in the endosperm of grains that have closed creases), but these are not involved in the lack of reversibility referred to above. Twenty percent of the grains in Manitoba wheat have exceptional looseness of structure in the dorsal endosperm, associated with a distinct delineation of the prismatic endosperm cells and an abnormally low density.

In Part I (3) the rates of change of density with variation in moisture content of dissected particles of endosperm and of semolina originating from the same wheat were compared. Density values at low moisture contents related to material dried from moisture levels not higher than 15%. In the present paper the behavior of endosperm that has been dried after having been raised to higher moisture contents is considered.

[1] Manuscript received February 16, 1955. Contribution from the Research Association of British Flour Millers, St. Albans, England.

The extent to which change in density of whole-wheat grains is reversible on drying after moistening has been previously investigated. Sharp (11) found that the density of wheat grains was linearly related to moisture content between 8 and 16%, but when the wetted grain was dried the original value was not fully regained, the deficit increasing progressively with extent of wetting. Matveef (9) has recently obtained similar results with a series of hard and soft wheats over the moisture range of 12–17%. Milner and Shellenberger (10) applied radiography to detect cracks caused in the endosperm of vitreous Kansas wheat grains by wetting to 30% or more, followed by air drying at 35°C. No cracks were detected after wetting only to 20%, followed by drying at 25° or 35°C., but the density of the dried grain was lower, by as much as 0.03, than its original value.

Materials and Methods

Wheat and Semolina (sizings or coarse middlings). The materials used were those described in Part I.

Peeled half-grains were prepared by bisecting Manitoba wheat grains longitudinally and carefully scraping away the bran and germ by means of a scalpel while the specimen was held under low-power magnification. The average weight of the "peeled half-grains" so obtained was 15½ mg.

Moisture contents and densities were determined by the methods described in Part I. Moisture contents are expressed on the moist basis. In determining densities of whole-wheat grains, suspension in the mixed organic liquids was effected under vacuum. The procedure for adjusting the moisture content of semolina and of individual particles was described in a previous paper (7).

Results

Reversibility of Density of Endosperm Particles. In Fig. 1, lines A and B are reproduced from Fig. 1 of Part I without the respective experimental points. The following symbols represent specimens dried slowly at 20°C. after being moistened to 20% and 23% respectively:

(20%) Hollow circles: dissected endosperm particles. S.*
Crosses: semolina, in bulk. D.*
Hollow triangles: semolina, individual vitreous particles. S.

(23%) Solid circles: endosperm moistened after dissection from grain at 12.6% moisture content. S.
Solid triangles: semolina, individual vitreous particles. S.

*S denotes that the density was determined by the method of suspension in mixed liquids; D, that it was determined by displacement of xylol in the pycnometer. The uppermost symbol in each set in Fig. 1 represents the specimen moistened to the limit in question without subsequent drying.

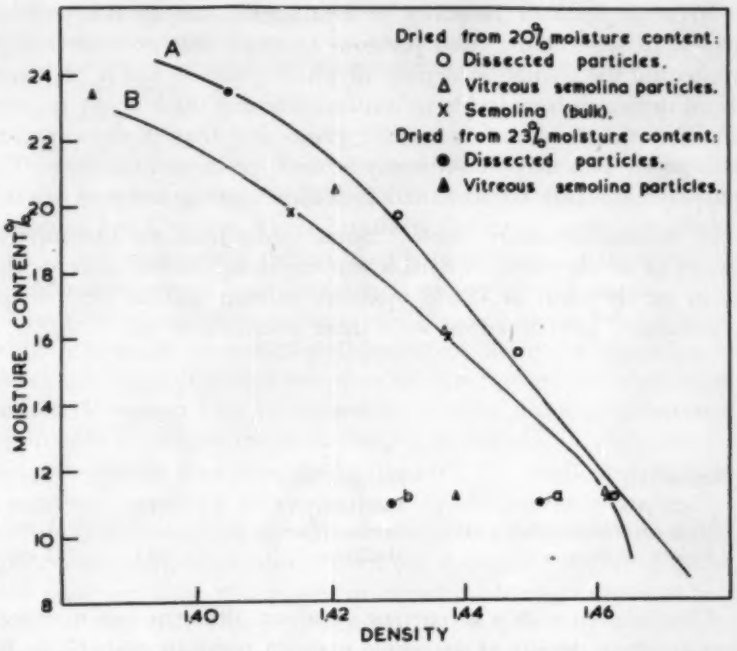

Fig. 1. Effect of moistening to 20% and 23%, respectively, and of subsequent drying, on the density of endosperm particles of various types. Lines A and B are reproduced, without the respective experimental points, from Fig. 1 of Part I (3).

The hollow circles, for endosperm particles dried after dissection from grain at 20% moisture content, lie close to line A, and the crosses (for semolina) and the two lowermost hollow triangles (for vitreous semolina particles) lie close to line B. That is, the change in density of endosperm fragments, caused by moistening them to a limit of 20%, is fully reversible on subsequent drying, irrespective of the mechanical history of the fragments.

The solid symbols in Fig. 1 show clearly, however, that when endosperm is moistened to 23% the original density is not fully regained on drying. Broadly speaking, the degree of expansion, or loosening of structure, that this implies is characterized by the bending over of the upper portions of lines A and B. Visually, it is associated with an intensified development of mealiness (or rather, turbidity) in the fragments. The extent of the failure to regain the original density is in step with the degree of visual change; thus, the points *a* and *b* in Fig. 1 refer to particles showing, respectively, least and greatest development of turbidity in the course of the moistening and drying treatments.

Relation between Densities of Endosperm and of Whole-Wheat Grains. In view of the cited previous findings, that even after slight moistening the change in density of whole grains is not fully reversible on drying, it is desirable to consider whether there is any relationship between the density of whole grains and that of the contained endosperm. This may conveniently be done under two headings: 1) at "natural" moisture content, and 2) with changing moisture content.

At Natural Moisture Content. Some values from the literature for density of whole grains of hard wheat are shown below, after recalculation on the basis of 12.6% moisture content and 25°/25° density specification, in comparison with those obtained by us:

Author (s)	*Wheat*	*Protein Content*	*Density*
		%	
Sharp (11)	Marquis, 1922	13	1.411
Sharp (11)	Marquis, 1923	17	1.388
Milner and Shellenberger (10)	Pawnee (Kansas)	...	1.370
Present authors	Manitoba	13.1	1.432

Clearly, even with a given type of wheat, different lots may differ appreciably in density of the whole grain. A previous study (7, p. 185) indicated little variation in endosperm density between vitreous grains of various types at a given moisture content, even though the protein content varied considerably.[2] This finding, however, related only to endosperm from the cheek centers. Arithmetically, on the basis of the above figure (1.432) for Manitoba grain and of values close to 1.30 for the densities of bran and germ (2, 4, 8), the density of the endosperm as a whole should be about 1.46, if the proportion of endosperm in the grain is taken as 85%.

As an experimental check on this calculation, the densities of six peeled half-grains, from the same wheat, were determined by the suspension method. The individual values ranged from 1.433 to 1.452, mean 1.445. This mean is appreciably lower than the calculated value and some of the individual values were very much lower. Moreover, the mean is lower than the values found for endosperm particles dissected from grains of the same wheat. Thus, for materials all at 12.6% moisture content, the respective values are as shown in the table following:

[2] In the case of grains that are not entirely vitreous the density of the whole grain has been shown, e.g., by Bailey (1), to increase with protein content. This is understandable because, with wheat of a given type, the proportion of mealy grains decreases as the protein content rises and the density of quite mealy endosperm is relatively low.

Endosperm particles dissected from cheek centers:	1.458[a]
Endosperm particles dissected from dorsal region:	1.453[a]
Mean value for dissected particles representing entire endosperm:	1.454[b]
Peeled half-grains	1.445
Whole grains	1.432

[a] From Fig. 2 of reference 7.
[b] Actually, 1.458 less 0.0037, as explained on p. 330 of preceding paper (reference 3).

The maximum individual value cited above for the peeled half-grains is 1.452, which is not far from the mean value for dissected particles of endosperm: the problem is to account for the abnormally low densities shown by a proportion of peeled half-grains.

In partial explanation, exceptionally low density in individual whole grains, or in peeled half-grains, of Manitoba wheat is often associated with abnormal looseness of structure of the endosperm in the dorsal region. This is characterized under low magnification by a prominent ridging of the dorsal region as exposed in a transverse section; the ridges represent the outlines of the needle-shaped or prismatic cells in which the endosperm in this region is formed. The feature is evidently associated with an unusual degree of intercellular space, since particles dissected from the dorsal endosperm of grains of this type are abnormally low in density, although those from the cheek are normal. This is illustrated by the following density figures obtained on specimens prepared from two Manitoba grains of 12.6% moisture content, of which one appeared normal in cross section while the other showed the prominent dorsal ridging:

	Normal	*Ridged*
Peeled half-grains	1.452	1.432
Particles from dorsal region	1.453	1.441
Particles from cheek center	1.458	1.458

The difference (0.005) between densities of endosperm particles from dorsal region and from cheek center of the normal grain is of the order previously reported (7, Fig. 2); in the case of the ridged grain it is more than three times as great.

Examination of cross sections of 50 grains taken at random from Manitoba sample A showed that this feature of dorsal ridging was present in about 20% of the grains. Although it may generally be found in Manitoba wheat, it appears not to have been described in connection with grain of natural moisture content, though Greer and Hinton (5) noted that when thin sections cut from vitreous grains of hard wheat were slightly moistened the outlines of the endosperm cells became distinct. Grains having the dorsal ridging might have been expected to break down exceptionally easily during milling, as a result of looseness of structure of their dorsal endosperm; evidently,

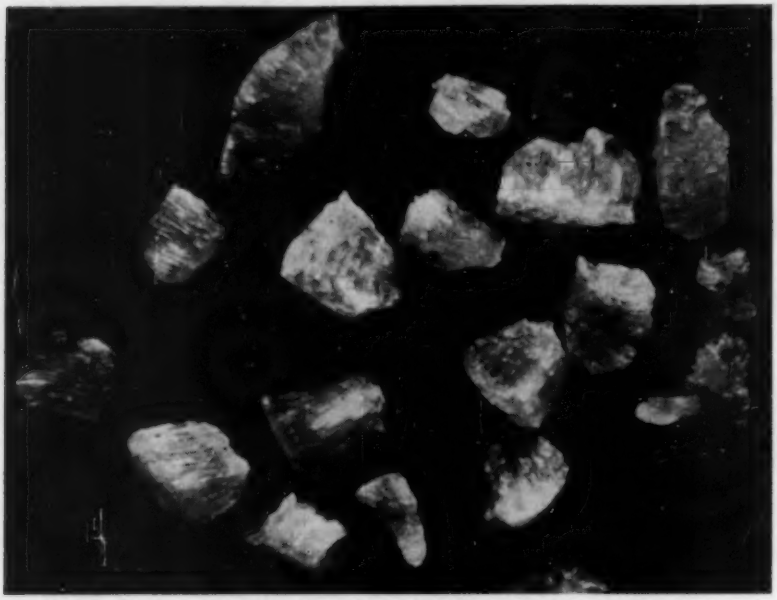

Fig. 2. Particles of semolina (sizings) with exceptionally distinct appearance of prismatic endosperm cells, associated with abnormally low density. 16×

however, this does not occur, at any rate during breaking, because, as previously reported from these laboratories (6, Fig. 1), the ridged endosperm may be seen in some of the coarser particles (semolina) produced by the break rolls. Figure 2 shows a number of such particles of semolina. A count under low power magnification showed that, in semolina laboratory-milled from Manitoba A, 12% of the particles present were of this type. It should be added that abnormally low density of whole grains was not invariably associated with this feature; in some cases the dorsal endosperm appeared normal but irregular hollow spaces or clefts in the endosperm were found on sectioning.

With Changing Moisture Content. Figure 3 reproduces parts of lines A and B from Fig. 1. The other continuous lines show the changes in density with increasing moisture content for C, peeled half-grains raised, by means of vapor equilibration, to 22% moisture content; D, peeled half-grains raised to 18.5% moisture content; E, whole-wheat grains raised to 18.5% moisture content. The corresponding discontinuous lines show the density changes on slow drying of the materials to their original moisture content, by exposure to still dry air at 20°C. Lines C, D, and E were drawn through points representing averages of density values (determined by suspension) on six indi-

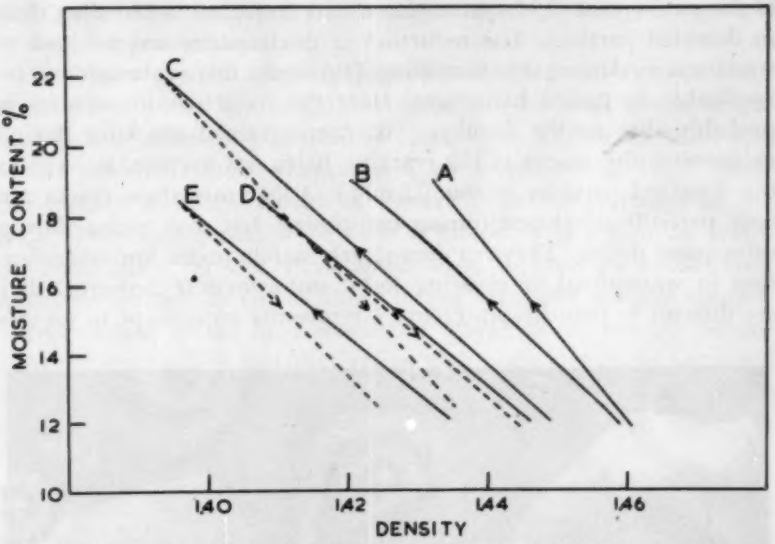

Fig. 3. Effect of moistening (shown by continuous lines) and of subsequent drying (discontinuous lines) on the density of: C, peeled half-grains moistened to 22%; D, peeled half-grains moistened to 18½%; E, whole-wheat grains moistened to 18½%. Lines A and B, for particles of dissected endosperm and of semolina, respectively, are reproduced from Fig. 1.

vidual specimens, the change in moisture content of which was determined by direct weighing. The whole grains were suspended in liquid under vacuum so it is unlikely that the crease plays any part in the phenomenon discussed.

Lines E of Fig. 3 show that, on drying after moistening only up to 18.5%, the change in density of whole-wheat grains is not fully reversible, a finding in keeping with that of the earlier workers cited. The peeled half-grains, however, show a much smaller deficit in density on drying after moistening to 18.5%, though when they are dried after moistening to 22% the deficit is relatively great; the difference in effect of the two degrees of moistening is in some measure like that shown in Fig. 1 for endosperm particles. The small deficit with the peeled half-grains dried from 18.5% suggests that the nonreversibility shown by the whole grains is substantially due to some change in bran structure; modification in endosperm properties contributes substantially to the effect only when the moisture content is raised above 20%.

The slope of the ascending (continuous) lines, both for peeled half-grains and for whole grains, resembles that of line B rather than line A. It was suggested in Part I that the difference in slope of lines A and B indicates that the capacity of the endosperm to share in contraction

of the total system (endosperm plus water) is smaller in semolina than in dissected particles. The reduction in this capacity was ascribed to structural weakening due to milling. Obviously, this explanation is inapplicable to peeled half-grains. Here the reduction in capacity is probably due to the incidence of coarse-grained cracking during moistening, the tessera of the cracking being, on average, as large as the dissected particles (about 0.5 mm.). Apparently these cracks are only partially developed during moistening, but may widen during subsequent drying. They are then clearly visible under low magnification in transmitted or glancing light; unfortunately, however, they are difficult to photograph. Figure 4 represents an attempt to do this

Fig. 4. Peeled half-grains showing coarse-grained cracking as a result of moistening to 20% followed by drying back to original moisture content (12.6%). Examples of cracks are indicated by the arrows. 7×

in the case of specimens dried gently after having been raised to 20% moisture content, but only a few of the many cracks that were clearly visible to the aided eye are distinguishable: they are indicated by means of arrows. Comparison of their spacing with the apparent length of the grains (actually, about 5 mm.) shows that they are roughly 0.5 mm. apart. In many grains (not shown in the photograph) the cracks were observed to run at right angles to the length of the grain, generally at intervals of 0.3–0.7 mm. These cracks appear to be of the type described by Milner and Shellenberger (10) in whole grains dried after being wetted to 30% or more. These authors did not, however, detect them by their radiographical method in grain that had been wetted only to 20%. Again, as already described, moistening above

20% causes the development of mealiness, which may be regarded as very fine-grained cracking.

The coarse-grained cracking referred to occurs in all vitreous Manitoba grains under the conditions described. In some grains coarse cracks of a different type (radial cracks) are visible in transverse section when Manitoba wheat has been moistened to 17%. These run radially from the trough of the crease to the periphery of the grain. They may generally be seen as in row B of Fig. 5, in grains the inner cheek faces of which touch so as to close the crease; such grains formed a majority of those in the Manitoba samples studied. Corresponding grains in unmoistened wheat, as in row A, do not show the cracks. The fact that grains with open creases do not crack in this way after moistening (row C) suggests that expansion of the endosperm occurs in a ringwise manner on moistening; in grains

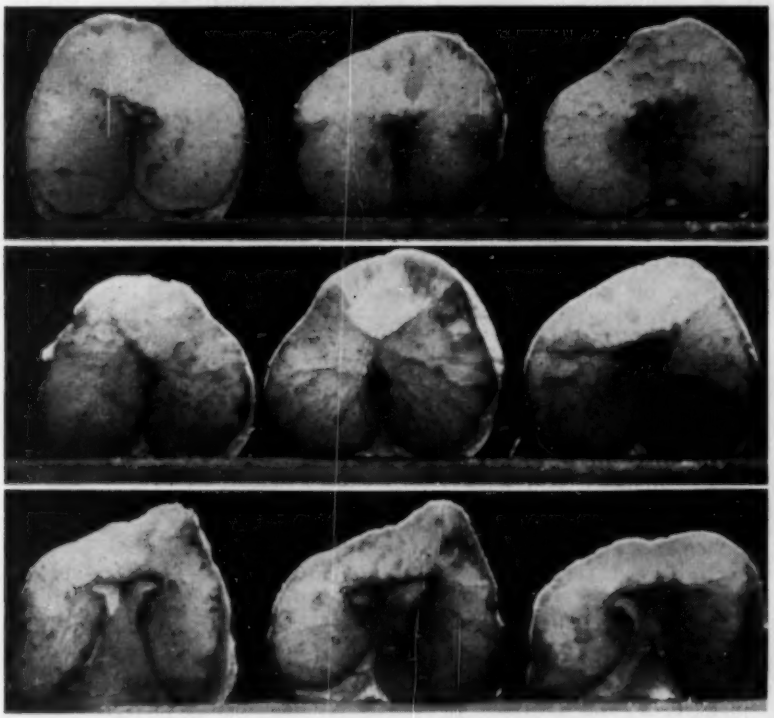

Fig. 5. Cut surfaces of whole-wheat grains dissected transversely to show radial cracking on moistening to 17%. A, at natural moisture content: no cracking. B, cracks showing in moistened grains where cheek surfaces are in contact. C, no cracking in moistened grains where cheek surfaces are still apart. 10×

with closed creases the resulting strain is evidently relieved by the radial cracking.

On the general subject of endosperm cracks it should be added that, irrespective of the distance apart of the cracks, their width must bear on the present considerations. Since density has been determined by displacement, presumably cracking of a particle can result in a decrease in density only when the cracks are impenetrable by the liquid within the experimental period. It is well recognized in studies of porous materials that pores of a certain diameter may permit penetration by water but not by nonpolar liquids, and the present phenomena are probably based on a mechanism of this kind. During the moistening (by water) the cracks can hardly have become penetrable by the organic liquid; otherwise they could not have caused the density fall of the peeled half-grains to be greater than that of the dissected particles, in the way suggested. Again the intensification of mealiness, in dissected particles, corresponding with the increase in rate of density change above 20% moisture content, evidently represents the development of a system of cracks too fine to be readily penetrable by the organic liquid. Further consideration of this aspect of crack width must, however, be postponed.

Acknowledgments

The authors are indebted to Dr. J. J. C. Hinton, who kindly took the photographs shown, and to Drs. J. B. Hutchinson and N. L. Kent for advice on statistical treatment.

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BOOK REVIEWS

Standard-Methoden fur Getreide, Mehl und Brot. Edited by the "Arbeitsgemeinschaft Getreideforschung." 38 pp. Verlag Die Muhle, Detmold, Germany, 1954. Price: DM 9.80.

This booklet contains fifteen tests for quality and other characteristics of grain, flour, and baked products. It is addressed mainly to quality control technicians, but all cereal chemists will find it useful at times; especially for understanding the publications of the "Arbeitsgemeinschaft," since these are some of the methods used in their work.

Methods for the following determinations are described in detail: Moisture, ash, crude protein, fat, maltose number, starch, degree of acidity, foreign matter in grain, wet gluten, and test number. Also covered are baking tests for wheat and rye flours and fat determinations in baked goods.

A method which will be new for some is the "test number," also known as the "Pelshenke test," which is the time in minutes a yeast-raised dough ball will float on the water surface prior to its disintegration. High test numbers indicate good gluten properties; e.g., good extensibility and elasticity.

The baking tests are especially designed for bakery practices where relatively weak flours and lean formulas are used to produce crisp, hard rolls and hard-crust bread demanded by central European consumers. Valuable suggestions for evaluating baking tests can be found in the corresponding sections.

The thorough and complete description of necessary steps in every procedure listed should make it easy even for inexperienced technicians to obtain satisfactory results. The majority of tests are simple, and the required reagents and apparatus are normally available in any quality control laboratory.

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Outlines of Enzyme Chemistry, by J. B. Neilands and Paul K. Stumpf. x+315 pp.; indexed. John Wiley & Sons, Inc., New York, N.Y. Price, $6.50.

This text departs strongly from the more classical treatment of enzymology and gives emphasis to those aspects pertinent to cellular metabolism. The sections of the book are: general principles, physical chemistry, types of coenzymes and enzymes, and metabolic patterns. No attempt is made to give details about many individual enzymes. Half the text concerns general principles and physical chemistry of enzymes. The section on metabolic patterns gives discussion of glycolysis, the hexose monophosphate shunt, tricarboxylic acid cycle, oxidative phosphorylation, fatty acid oxidation, and synthesis of enzymes. Topics presented include recent material, such as the functions of coenzyme A and methods of detecting enzyme-substrate compounds. Useful and relatively liberal reference citations are made. The chapter on enzyme synthesis, although a good presentation of this subject, represents a detailed consideration of a topic where basic information is still lacking. The space might have been used for more adequate presentation of other topics.

Some important aspects of enzymology receive little or no treatment. For example, except for a classification table, practically no information is given about carbohydrases, lipases, proteinases, and peptidases. Thus the book would not serve for the reader interested in the enzymic degradation and synthesis of polysaccharides. There is no material on enzyme technology.

The book will serve a useful purpose for those who want a brief modern survey of enzyme fundamentals and the participation of enzymes in intracellular metabolism. The material given is clearly presented, and commendably free from errors.

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Cereal Chemistry

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References are arranged and numbered in alphabetical order of authors' names and show author, title, journal, volume, first and last pages, and year. The list is given at the end of the paper. Reference numbers must invariably be cited in the text, but authors' names and year may be cited also. Abbreviations for the names of journals follow the list given in *Chemical Abstracts* 45: VII–CCLV (1951).

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All drawings should be made about two to three times eventual reduced size with India ink on white paper, tracing linen, or *blue*-lined graph paper; with any other color, the unsightly mass of small grid lines is reproduced in the cut. Lettering should be done with a guide using India ink; and letters should be 1/16 to 1/8 inch high after reduction.

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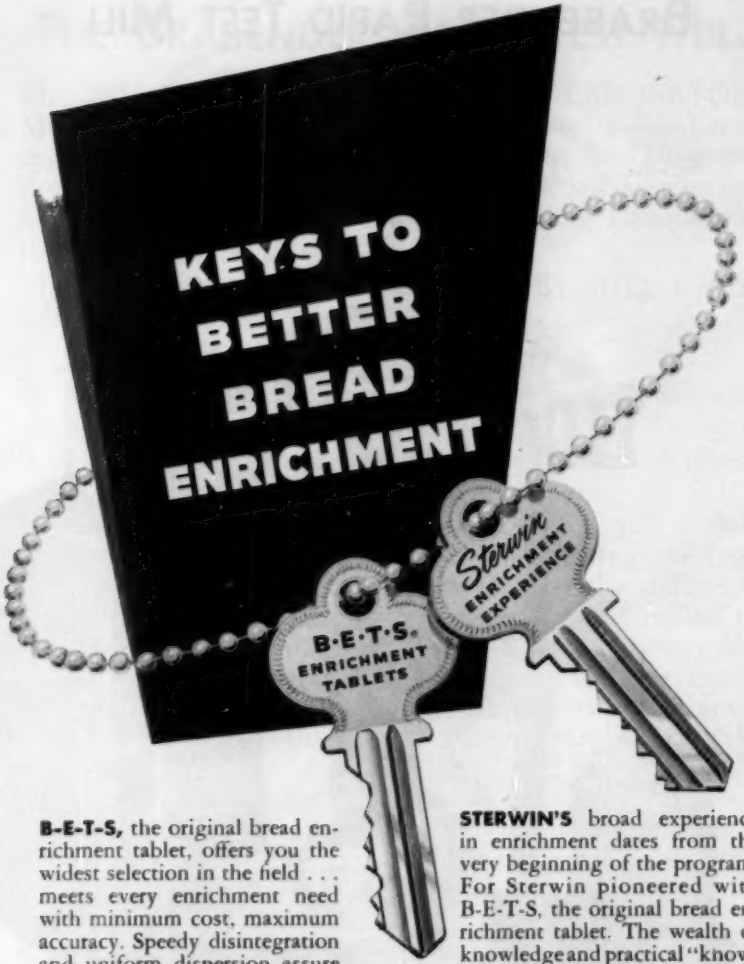

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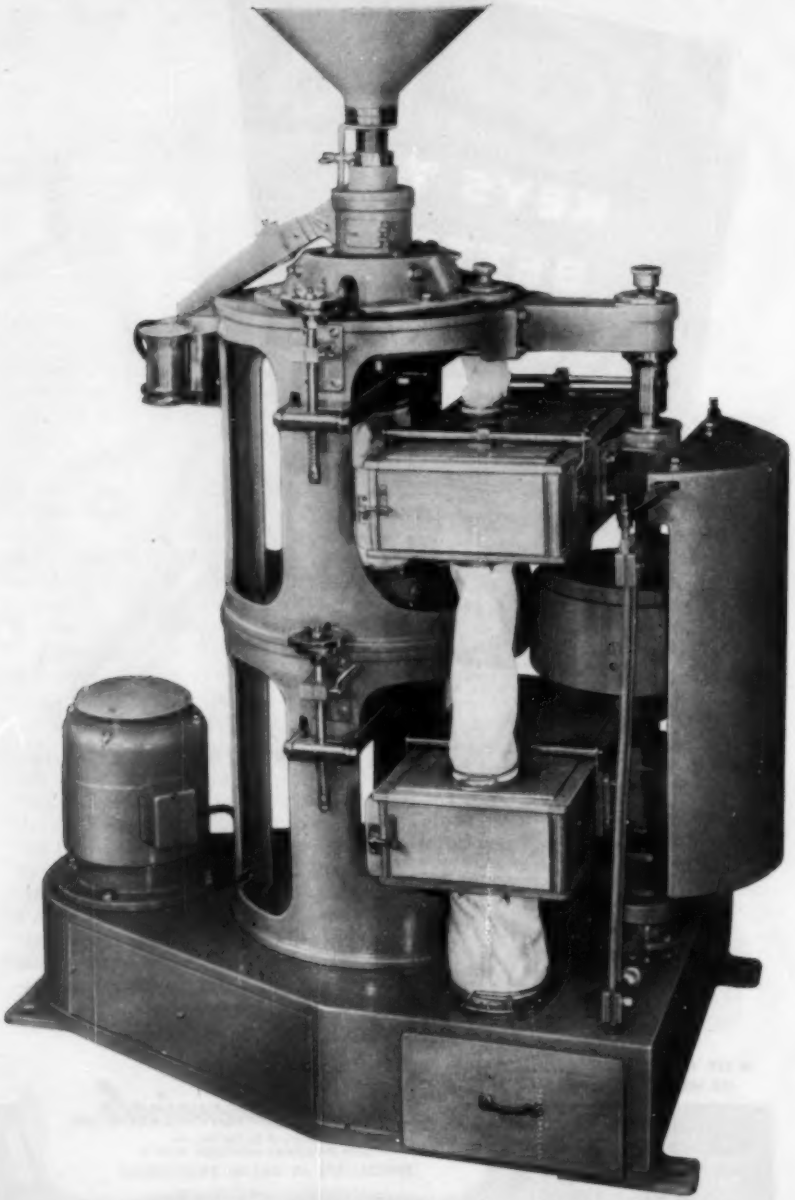

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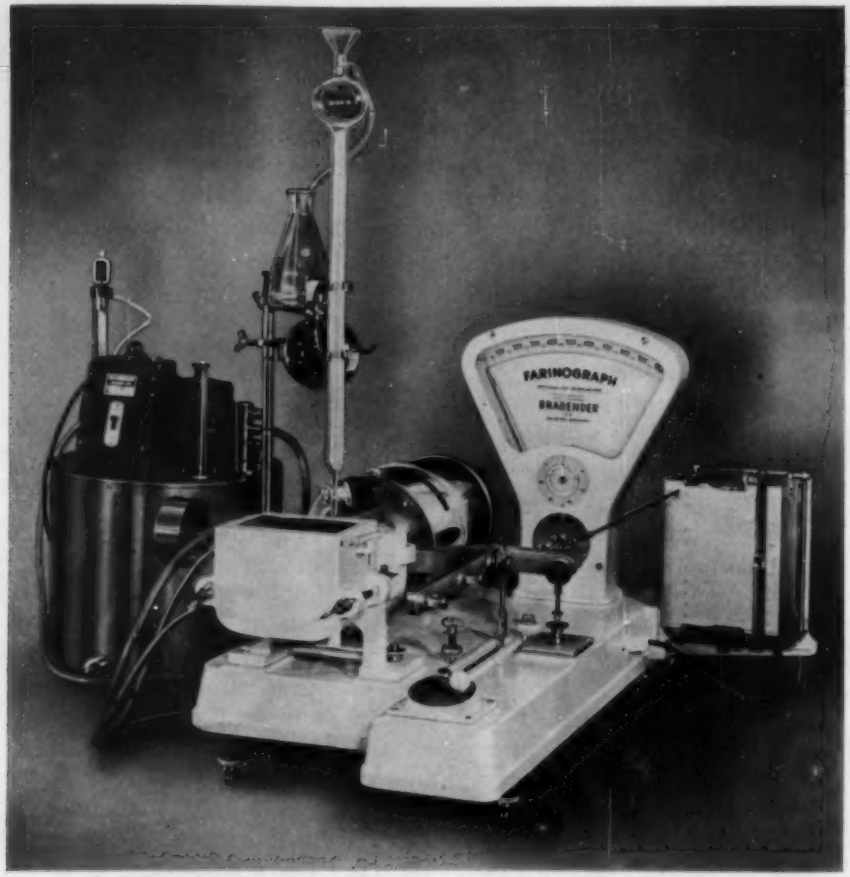
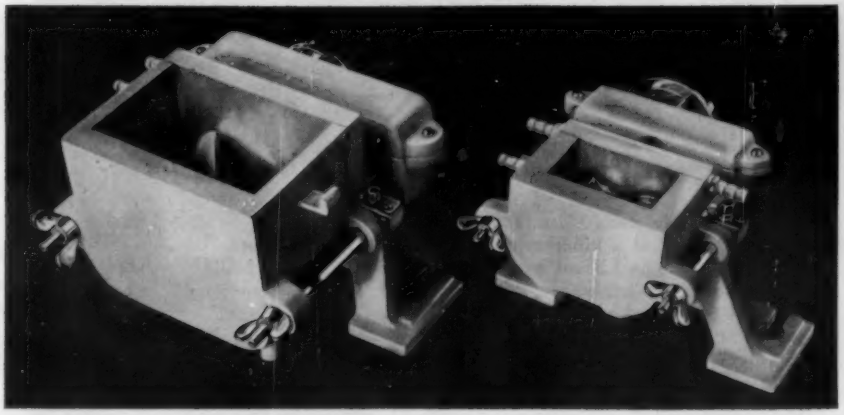


The genuine BRABENDER FARINOGRAPH 1956-model features:

1. The newly designed synchronizer phase-converter producing four times the torque of the present Farinograph at both speeds. It gives the Farinograph-Dynamometer the characteristics of a synchronous motor making it independent of voltage fluctuations and thereby assuring high calibration stability.
2. New Pressure Thermostat making extra cooling units (for hot climate) in 99 of 100 cases unnecessary.



Senior Mixer—300 grams **Junior Mixer—50 grams**

3. 300 grs. as well as 50 grs. mixers in both bronze and stainless steel available.

   Calibration of both bronze and stainless steel mixers can be supplied at your request on the basis of world standard established 1934 or any standard you may designate. Ask for our suggestions.

   NEW: We guarantee stainless steel mixers to give the same curves as bronze mixers through exclusive design. Patent applied for.

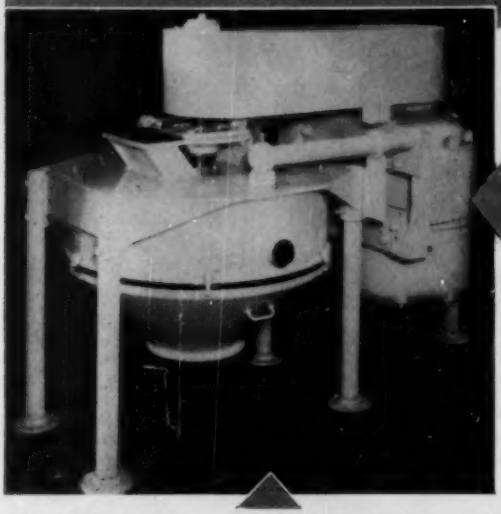
   All genuine Brabender Instruments and mixers are available on either purchase or rental basis. Ask us for attractive price quotations and new literature.

MIAG NORTHAMERICA, INC.
420 Flour Exchange Building, Minneapolis 15, Minnesota
Telephone: FIllmore 5713

Exclusively franchised Sales and Service Agents for genuine C. W. Brabender designed instruments as made by

BRABENDER OF DUISBURG (Western Germany)

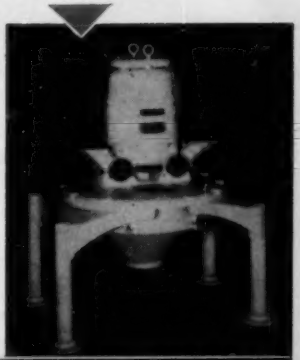
500 CWT PER HOUR!!!
it's a cinch with the NEW "BIG INCH"....



for Positive Infestation Control . . .

the NEW "ENTOLETER" 'BIG INCH' for 150 to 500 cwt per hour

the STANDARD "ENTOLETER" unit for 25 to 150 CWT per hour



You guarantee your flour specifications... let "ENTOLETER" Infestation Control assure the complete cleanliness of all ingredients and finished products . . .

. . . the trademark "ENTOLETER" on milling equipment is your guarantee of complete satisfaction. Write to us for information concerning our products. ENTOLETER DIVISION, The Safety Car Heating and Lighting Company, Inc., 1153 Dixwell Ave., New Haven 4, Connecticut.

The trademark "ENTOLETER" is your guarantee of complete satisfaction

ENTOLETER
CENTRIFUGAL MACHINES

Get this bonus with PY-RAN

At left, the circled tenth biscuit symbolizes the extra yield obtained from self-rising flour leavened with Monsanto PY-RAN.*

At right, nine biscuits represent the limited yield when an ordinary calcium phosphate is used.

When you use Monsanto's PY-RAN in your prepared mixes and self-rising flours, your customers get greater yield from your product. This means more repeat sales for you.

Above, you see an extra biscuit, an actual bonus, resulting from PY-RAN'S superior leavening action. PY-RAN (anhydrous monocalcium phosphate) holds its leavening action for release *in the oven.* That's why it gives such light, fluffy baked goods. PY-RAN has extra-long shelf life, too, because of a special moisture-absorbent coating that protects it from reaction during storage.

You'll like PY-RAN. It blends well with other leavening acids, gives attractive texture, crust and crumb color, reduces tunneling. You'll also like SAPP-40 for machine doughnut mixes; SAPP-28, a slow-action baking acid, and HT* Phosphate (MCP monohydrate).

Phone the local Monsanto office for the book "Monsanto Phosphate Leavening Agents," or write: Monsanto Chemical Company, Inorganic Chemicals Division, 710 North Twelfth Boulevard, St. Louis 1, Missouri.

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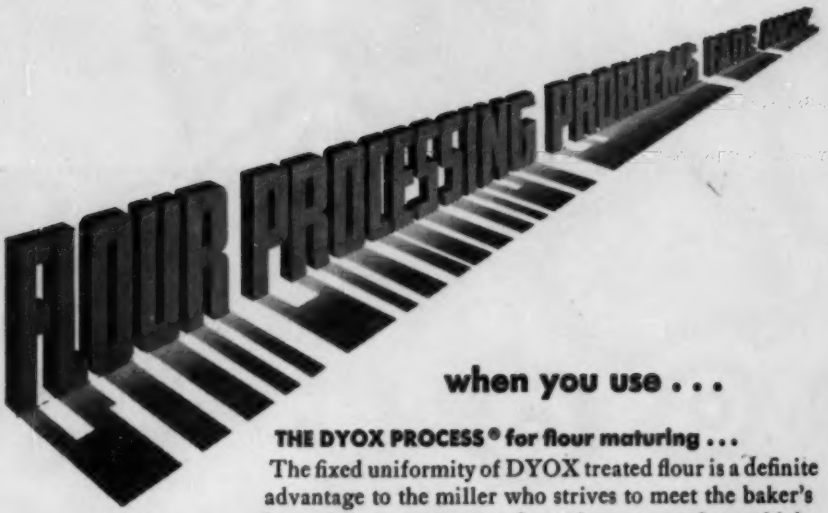

when you use . . .

THE DYOX PROCESS® for flour maturing . . .

The fixed uniformity of DYOX treated flour is a definite advantage to the miller who strives to meet the baker's demand for a starting product of constant value—which works in the bake shops with the least trouble and manipulation by the operator. DYOX treated flour assures overall dependability, uniformity of product, and ease of operation.

"NOVADELOX"® for whiter, brighter flour . . .

"Novadelox" is designed to meet the desire of the consuming public for a bread that is truly white—and to enable millers to produce a uniform product of standard color.

"N-RICHMENT-A"® for uniform enriching . . .

"N-Richment-A" provides a readily available, simple and troublefree product for enriching flour—with the assurance that the standard procedure of adding "N-RICHMENT-A" will produce a dependable standardized flour.

THE N-A FLOUR SERVICE DIVISION with more than a quarter-century of experience . . .

The N-A Flour Service Division with its skilled laboratories and staff is always available to work with you or your consultants on all phases of maturing, bleaching and enriching. Why not phone your nearest N-A Representative today!

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